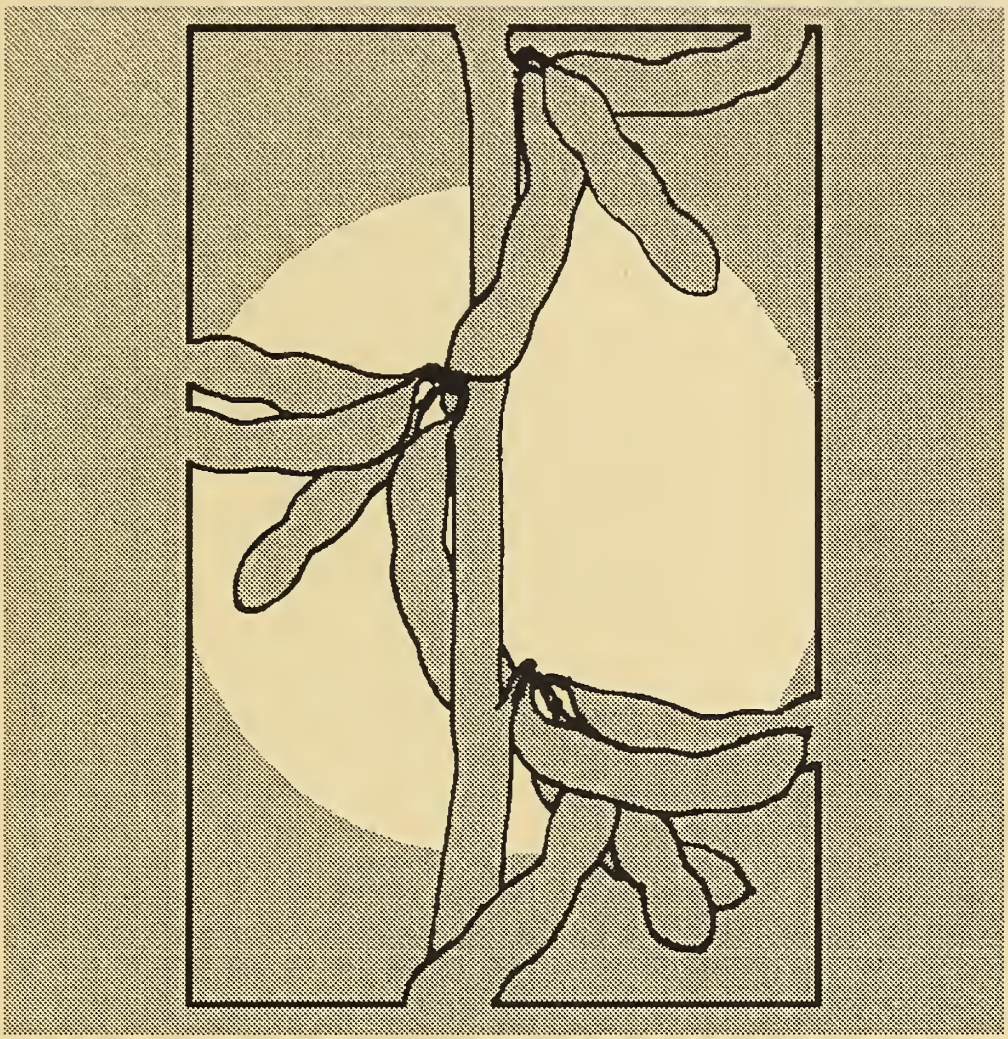
Soybean Genetics Newsletter



Volume 24 May 1997

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Department of Agronomy
and Department of Zoology / Genetics
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Foreword

Volume 24, 1997 of the Soybean Genetics Newsletter has been possible by the concerted effort of Tracy Dang, USDA ARS CICGR Unit, and Ivy Andersen, a recent graduate (Entomology major) of Iowa State University.

Several articles deserve special mention:

We sincerely appreciate our international and U. S. contributors. We look forward to your continued cooperation.

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Soybean Genetics Newsletter Homepage
http://www.public.iastate.edu/~rpalmer/Soybean Genetics.html

You can now visit our newly constructed homepage. Browse the table of contents and query of authors and articles for volume 1 (1974) to current volume. The home page is under continuing construction, and is updated weekly.

WORLD SOYBEAN RESEARCH CONFERENCE VI

CHICAGO, ILLINOIS, USA

AUGUST 1999

(Specific date and hotel to be announced later)

The Sixth World Soybean Research Conference will be held in Chicago, Illinois, USA in August of 1999. The University of Illinois at Champaign-Urbana and the National Soybean Research Laboratory will be responsible for organizing the conference. Both invited and contributed papers will be presented in program areas which range from Crop Improvement (Germplasm, Breeding, and Biotechnology), Crop Management (Tillage, Fertility, Integrated Pest Management, and Physiology), Processing and Utilization (Animal Feed, Human Food, Nutrition and Health, and Industrial uses), and Strategy (Marketing, Trade, Research and Development, and Information Technology).

Specific announcements and a call for papers will be circulated when arrangements for hotel and dates have been finalized. For additional information you may contact :

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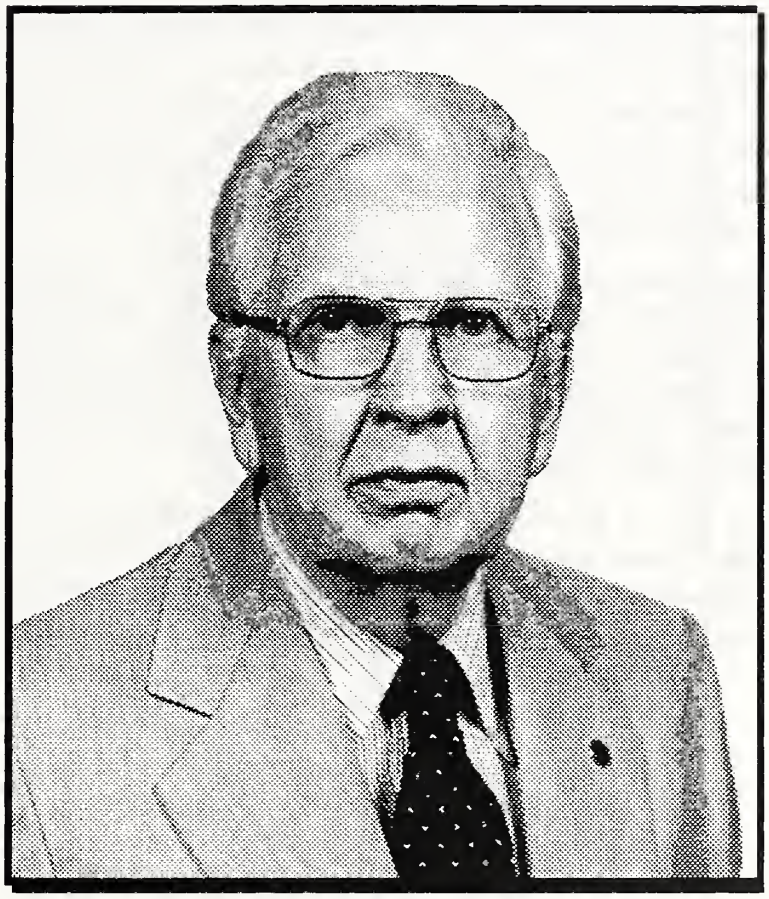
IN MEMORIAM

Dr. Edgar E. Hartwig: August 26, 1913 - May 11, 1996



Edgar Emerson Hartwig was one of the most widely known and successful soybean breeders in the world. Dr. Hartwig was respectfully and appropriately referred to as "The Father of Soybeans in the South". From the beginning of his work with soybeans in 1943, Dr. Hartwig played a major pioneering role in causing soybeans to ascend from an insignificant position among several forage crops to one of the most important U.S. grain crops.

He established his leadership in soybean breeding as early as 1954 with the development of the variety `Lee'. Lee quickly became the leading variety in the south. Early in his career, Dr. Hartwig recognized the need for pest resistance in developing higher yielding soybean varieties. Many of the 28 multiple-pest-resistant varieties, and 14 germplasm lines he developed and released, had resistance to the soybean cyst nematode, root knot nematode, and major diseases in the south. The varieties that Dr.

Hartwig developed and released were evaluated under a wide range of environmental conditions in the Cooperative Uniform Soybean Tests, Southern States. Dr. Hartwig served as coordinator of this program from 1948 through 1991. He also recognized the importance of preserving a diverse gene pool, and served as curator of the later maturing accessions of the Soybean Germplasm Collection for over 30 years. He frequently sampled this gene pool for useful traits to transfer to adapted lines, and then made these enhanced breeding lines available to other soybean breeders. There is little question that one of Dr. Hartwig's major contributions to soybean breeding in the south was the generous sharing of his breeding lines with other soybean breeders, public and private.

Dr. Hartwig published more than 150 refereed articles, including several book chapters. He was also a frequent contributor to popular articles in publications such as Soybean Digest and Delta Farm Press. He was very active internationally, as an invited speaker at conferences, where he shared his vast knowledge of soybean breeding and production with other scientists. Nationally, he was a very active participant in the American Society of Agronomy Meetings and the Soybean Breeders Workshop, which was his favorite meeting. He found in this setting an opportunity to share knowledge with those who had the same zeal for improving the soybean plant that he had.

For his dedicated service and significant contributions to science, Dr. Hartwig received numerous awards and honors. Among these awards and honors are the USDA Superior Service Award, American Soybean Association Honorary Life Membership, ASA and CSSA Fellow, USDA Distinguished Service Award, University of Minnesota College of Agriculture Outstanding Achievement Award, Mississippi State University Distinguished Professor, ASA Agronomic Achievement Award-Crops, and the ARS Hall of Fame Award.

Edgar Hartwig was a gifted gentleman who, with diligence, persistence, and hard work, dedicated his entire life to improving the soybean plant and increasing yields and profits from soybeans. He will be fondly remembered by the many scientists who looked up to him as their mentor, and by all those with whom he generously shared the soybean genetic stocks he developed.

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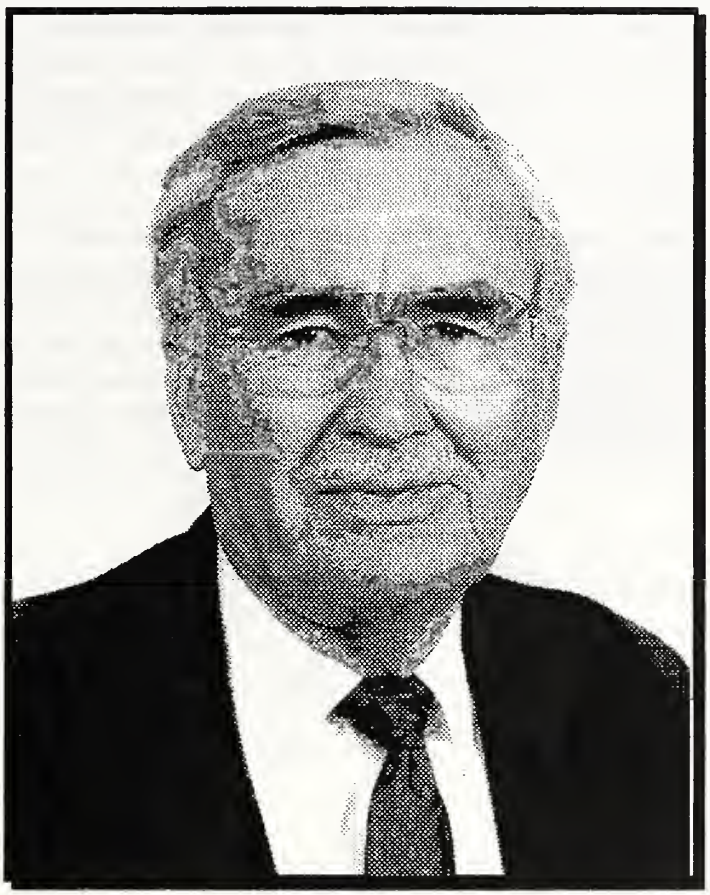
IN MEMORIAM

Dr. Kuell Hinson: 1924 - 1996



Southern soybean breeding recently lost one of its founding fathers with the death of Kuell Hinson. Dr. Hinson passed away at his home in Gainesville, Florida last October, shortly after being diagnosed with pancreatic cancer.

Kuell Hinson was born in 1924 in the small town of Moss, Tennessee. He served in the Army Air Corps during World War II, and then pursued his education; first at Tennessee Technological University, and then at the University of Wisconsin, where he received his Ph.D. degree in 1954. In 1955, Dr. Hinson came to Florida to begin a breeding program for soybean with the USDA.

Over the next 40 years he developed improved soybean varieties which came to be grown over substantial areas of the southern US, as well as South America and Asia. His work was instrumental in the development of varieties with resistance to root-knot nematodes, a major pest of soybean in tropical

and sub-tropical areas. In later years much of Dr. Hinson's research focused on the manipulation of photoperiod sensitivity in soybean, developing types with broad adaptability to various planting dates. Germplasm developed by Dr. Hinson is widely dispersed throughout soybean breeding programs worldwide, and many of these programs are directed by his former graduate students. His contributions to soybean breeding were frequently recognized through awards given by many organizations, including the Commercial Soybean Breeders.

As a graduate student of his, I came to respect Dr. Hinson's insight very quickly. He was a very serious teacher, and would always challenge his students to question what the data really. meant. His most frustrating, yet ultimately enlightening comment on a manuscript was "maybe you should think about this again". This element of self-discovery was a common thread in his teaching. I remember well being in the field with him and he smiling at my joy of "discovering" that growth habit and pubescence color segregate independently. This ability to let others discover what he already knew helped instill a love of the profession that continues today. And isn't that the ultimate goal of training the next generation of scientists?

Many of you who have come each year for many years to the Soybean Breeders' Workshop have already noticed Dr. Hinson's absence. He was physically tall and an imposing presence wherever he was, but never overbearing. His absence is a reminder to us that all things must pass. We sometimes forget that the time and efforts we devote to this profession will come to an end. But the good works we do will live long after us. I have seen the good works of Kuell Hinson alive in places like Mississippi and Georgia, but also in Argentina and Brazil. Kuell Hinson made a difference. Kuell Hinson was a soybean breeder.

Chris Tinius

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USDA SOYBEAN GERMPLASM COLLECTION REPORT

February 1997

In 1996, a total of 33,569 seed lots representing 12,287 different accessions were distributed from the USDA Soybean Germplasm Collection in response to 345 requests. Of these, 293 orders with a total 26,879 seed packets were sent to researchers from 31 states and 2,647 to Canada. The remaining were sent to scientists in 23 other countries. Over 22,000 packets were distributed for disease, nematode, or insect resistance screening. The largest efforts were evaluating for resistance to *Sclerotinia* whitemold (12,000 packets), aerial web blight (4,500 packets), *Phytophthora* rot (1300 packets), various nematodes (800 packets), soybean mosaic virus (750 packets), sudden death syndrome (700 packets), brown stem rot (700 packets), *Septoria* brown spot (700 packets), and *Rhizoctonia* rot (400 packets). Seeds of 1536 accessions were sent to the National Seed Storage Laboratory for backup.

Plots planted for seed replacement of *G. max* accessions in 1996 consisted of 1535 four-row plots planted at three locations: 1304 plots at Urbana, 204 plots at Stoneville, and 27 plots at Isabela, Puerto Rico. Seed replacement plots for *G. soja* accessions were planted at both Stoneville (75) and Urbana (49).

A second four-year germplasm exchange agreement was signed in 1996 by the USDA and Chinese Ministry of Agriculture. Under the terms of this agreement the collection will receive an additional 1,000 accessions. In the spring of 1996, we received the first 500 accessions from eight provinces of south China. In the previous two exchanges, we received accessions from four of these provinces (Fujian, Anhui, Sichuan, and Hunan) but had not received accessions from the others (Jiangxi, Guangxi, Yunan, and Guizhou). In May, 154 accessions were planted at Stoneville and 346 accessions were planted in Isabela in November. The following table summarizes the history of acquisition from these provinces.

Accessions in the USDA Soybean Germplasm Collection
received from eight provinces in south China

Province	Before 1980	1980-1992	1992	1994	1996	Total
Fujian	8	2	0	48	30	88
Jiangxi	1	12	0	0	30	43
Guangxi	0	0	0	0	60	60
Anhui	0	1	66	75	41	183
Sichuan	3	11	0	157	83	198
Hunan	0	18	0	12	27	57
Yunan	0	0	0	0	118	118
Guizhou	0	0	0	0	111	111

We also received 267 additional new foreign *G. max* accessions. These introductions came from Japan (192), South Korea (40), other sources in China (18), Ukraine (15), and Mongolia (1). All but 26 of the accessions received in 1996 were planted last year at either Urbana or Stoneville. There was only one *G. soja* that was received from Japan. We also received seed of 31 domestic cultivars, germplasm releases, and genetic types.

After purelining in 1996, 20 *G. max* accessions were added to the collection from China (14), Japan (1), Indonesia (4), and Thailand (1).

General field evaluation of 1253 recently acquired accessions in maturity groups V through VIII was begun at Stoneville, Mississippi. Field and seed data for all evaluations conducted from 1992 through 1995 have been entered into the computer. These include the 1992-93 MG VI evaluation at Stoneville; the 1994-95 MG 000 - I evaluation at Rosemount, Minnesota; the 1994-95 MG I - IV evaluation at Urbana; the 1994-95 MG

VII - VIII evaluation at Stoneville; and the 1992-95 MG IX and X evaluations in Puerto Rico. These data will be published in USDA Technical Bulletins and also made available through the GRIN.

Improvements in collection operation achieved this year include the ability to weigh seed and automatically enter seed weights (100 seed and total) directly into the database. When all samples have been weighed, these data will be used to monitor seed inventory and determine when accessions need to be grown because of low supply. All origin data in the database has been reviewed and corrected when necessary. Province of origin data is available for about 70% of the introduced *G. max* accessions and 85% of the *G. soja* accessions. This information also has been added to the database. The origin data now in place will be one of the key criteria in the selection of the core collection. The designation of the core collection is being delayed until the most recent acquisitions from China are added to the Collection so that all of the soybean growing provinces in China will be represented.

As of December 31, 1996, the Collection database contained data for the following entries:

Subcollection	**Entries**
Introduced *G. max*	14,379
G. soja	1,102
Germplasm releases	141
Modern cultivars	392
Old cultivars	208
Private cultivars	35
Williams isolines	100
Clark isolines	295
Harosoy isolines	134
Other isolines	35
Genetic types	150
Total	16,962

J.L. Hill and R.L. Nelson
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Soybean Crop Germplasm Committee Meeting

The Soybean Germplasm Committee held its annual meeting February 17, 1997 in conjunction with the Soybean Breeder's Workshop at St. Louis, MO. In attendance were committee members Tom Devine, John Hicks, Bob Freestone, Doyle Ashley, Earl Hendrix, Bill Kenworthy, Randall Nelson, Dwayne Buxton, Terry Niblack, Jerry Hill, Tom Kilen, Dan Phillips, Michael May, Jim Orf, Elroy Cober, Xavier Delannay, Emerson Shipe, and guests Tommy Carter, Mark Bohning, Scott Abney, Jeff Thompson, and Jeff Tomkins. Chair, Tom Devine, called the meeting to order at 8:00 a.m. New members Earl Hendrix, Michael May, Dwayne Buxton, Jim Orf, Xavier Delannay, and Elroy Cober were introduced. Tributes to Dr. Edgar Hartwig and Dr. Kuell Hinson were given by Tom Kilen and Emerson Shipe, respectively. Tom Devine distributed copies of a document which describes the function, duties, and responsibilities of crop germplasm committees.

An agenda was sent to the committee members prior to the meeting naming members to the four subcommittees and listing topics for discussion. The committee broke into subcommittees until 10:00 a.m.

Acquisition (Kenworthy, Chair; Ashley, Hicks, Nelson)

Germplasm exchange with China is proceeding with 1500 new accessions (*G. max*) received since 1992. All provinces in China are now represented in the collection. Breeders who would like to obtain specific cultivars from China should contact R. Nelson and attempts will be made to acquire the seed. A collection trip to Vietnam is being planned.

The number of private cultivars in the germplasm collection is relatively small. Not all public cultivars are placed in the collection because of intellectual property rights issues. Question was raised about the importance of having a complete set of public cultivars in the collection. Subcommittee of R. Nelson, Chair; Orf, Shipe, Kilen and Cober was appointed to study the issue and report to the committee.

A subcommittee of J. Orf, Chair; Nelson, Freestone, Niblack, and Phillips was appointed to work on developing a "Long Range Plan" for acquisition of germplasm.

Evaluation, Crop Vulnerability (Phillips, Chair; Niblack, Hendrix, Cober, All)

I. Maintenance and Distribution of Pathogen Collections Needed to Utilize Disease Resistance Genes Present in the Germplasm Collection

(A) Fate of the *Phytophthora* collection-To incorporate Phytophthora resistance into soybean cultivars, cultures of known races must be used to screen progeny. For several years, both public and private breeders have depended on three programs to provide properly identified and virulent cultures of specific races of this pathogen. The leaders of these projects have had neither a mandate nor specific funding to provide this invaluable service. Since the retirement (without replacement) of one of these leaders, Bobbie Keeling of Stoneville, this committee has warned that maintenance of this working collection was in danger. Now with the retirement of the second leader, Fritz Schmitthenner of Ohio, the situation becomes critical. This leaves only one active pathologist, Scott Abney of Indiana, maintaining a collection of identified, virulent cultures. Scott is working with *Phytophthora*, but has no specific mandate or funding to provide cultures to breeders. To provide the services needed by breeders, the entire *Phytophthora* collection should be maintained in at least two separate locations, staffed and funded to properly maintain and distribute the cultures and monitor the development of new races of the pathogen.

(B) Cyst Nematodes and other pathogens—A similar situation exists with regard to the availability for distribution of properly identified cultures of the cyst nematode. This is not a direct result of retirements, but is more from a lack of a mandate or funding for this expanding and very important activity. Collections of other soybean pathogens are not in crisis situations at present, but are threatened as downsizing of Universities and USDA-ARS continues.

Subcommittee Recommendation:

These are national problems that should be addressed on a national scale. Private breeders and soybean producers (United Soybean Board) have a great interest in this problem and have expressed a willingness to assist in funding a well planned solution to this problem. They, however, are not the agencies that can provide the long-term commitment needed to remedy this situation. The USDA-ARS is the agency best situated to provide this service, either through internal programs or through long-term support to Universities or other agencies.

The subcommittee urges that the full committee express to all levels of administration in USDA-ARS the urgent need to remedy the critical situation of the Phytophthora collection. The impending crisis in the cyst nematode collections and other pathogen collections should also be brought to their attention. Without these collections, the ever-increasing pool of valuable resistance genes cannot be identified or utilized.

Specific Recommendations Concerning the *Phytophthora* Collection

The subcommittee suggests that the USDA-ARS consider the following two actions: (1) Provide Scott Abney with a mandate and the necessary additional funding to collect and maintain for distribution to breeders and others, all available races of *Phytophthora* from soybeans. (2) Include maintenance and distribution of *Phytophthora* races as a major, properly funded part of the duties of the pending new position (Bobbie Keeling replacement) at Stoneville. Abney (USDA-ARS, Purdue University) could take the lead for distribution of the races prevalent in the North-central region and the Stoneville position for the races prevalent in the Southern region, but the entire collection should be maintained at both locations.

A continuing subcommittee (Phillips, Chair) is being formed to address the issue of maintenance and distribution of collections of pathogens that are critical to the utilization of disease resistance genes present in the germplasm collection. This subcommittee will address the maintenance and distribution of all working collections, but highest priority will be on resolving the critical needs relative to the *Phytophthora* and cyst nematode collections.

II. Evaluation of recent acquisitions

Seed of many of the recent Chinese acquisitions is being increased and reasonable quantities will be available for evaluations. Recent acquisitions, particularly the Chinese lines, are being evaluated for resistance to several diseases, nematodes, and insects in addition to yield, quality and other traits. As a result, these lines will soon become the most thoroughly evaluated group in the collection. This extensive testing is very important but has helped to create a problem with information flow to breeders. There is a "bottleneck" in getting the evaluation data entered into the computer, where it would be more readily available to breeders.

Subcommittee Recommendation:

The subcommittee requests that USDA-ARS provide additional funding, at least on a temporary basis, to get this backlog of important information entered and available to breeders.

Enhancement (Freestone, Chair; Buxton, Devine, Shipe, Orf, May)

The subcommittee discussed the questionnaire on "Gene-Pool Enrichment" from Dr. Kenneth Frey and Dr. Allan Stoner (National Plant Breeding Study). There was general agreement that the focus of the survey was quite broad. Shipe will coordinate the response with input from Freestone, Carter, Kilen, and T. Hymowitz.

One-page written reports were distributed and discussed from three current "enhancement" projects: (1) Soybean Asian Variety Evaluation (Carter), (2) Screening New Chinese Accessions for *Phytophthora* Resistance (Schmitthenner, St. Martin, Lohnes), and (3) Screening Maturity Group O - III PIs for Resistance to White Mold (Diers).

There was discussion of improved, more timely methods of communicating results of screening/evaluation tests to breeders. Suggestions included use of the internet and more use of the Soybean Genetics Newsletter.

A suggestion and rationale for revision of public cultivars/germplasm release notices were made. Intent is to make the information more "user-friendly" by establishing a tabular, standardized format. Freestone and Carter will work with other breeders and pursue this.

Operations (Kilen, Chair; Delannay, Hill, Bohning)

A core collection would aid scientists in selecting accessions for evaluation. In the past, the size of the Collection has not made the core collection a high priority. When the purelining of the accessions obtained from China in 1996 is completed, the Collection will have accessions from all soybean growing provinces in China. This would be an appropriate time to establish a core collection for *Glycine max*.

The GRIN system is constantly being made more user friendly. Links with databases in other countries are being developed. Development is continuing on a new version of PC GRIN. This version will be useful to other countries to provide a link to the GRIN and in managing their germplasm collections. The ability to store and display pictures is now available and is being refined. Pictures of soybean accessions are being solicited for addition to the database.

Within the next year, 1992 through 1995 data on agronomic data, protein and oil content of approximately 4,000 accessions (Maturity Group OOO-X) will be added to the GRIN system. There will be additional printed material on newer accessions, but that information will appear later than the same information on GRIN. Seed samples of the first and second set of recent China introductions are available in small quantities (50 to 100 seeds).

Adequate storage facilities exist at Urbana for the near term. Chemical dessication units have reduced relative humidity from 50% to 25%. This is expected to extend seed viability for periods greater than 10 yr for *G. max* and 15 yr for *G. soja*. At the current rate of Collection growth, the current storage facilities will be filled to capacity in ten years. Such an increase would also require additional technical personnel.

Other Business

Jerry Hill distributed the 1996 Soybean Germplasm Collection Report.

The recent GAO "Survey on The National Plant Germplasm System" which was completed by members of the various Crop Germplasm Committees was discussed.

The Soybean Crop Germplasm Committee Report was distributed. The report was a major focus of the 1996 Committee Meeting and was compiled and finalized by Randy Nelson.

T. Carter reported that a USDA Technical Bulletin which contains pedigrees of modern Chinese cultivars will soon be printed and available.

Mark Bohning distributed a progress report on GRIN. Pertinent points from this report are found under the Operations subcommittee report.

M. May stated that the U.S.B. recognizes the importance of the work and responsibilities of this committee. The U.S.B. is willing to assist if additional meetings are deemed necessary.

Appreciation was expressed to retiring members, Devine, Ablett, and Kenworthy.

Shipe was elected Chair for 1997. Orf was elected Vice-chair.

Meeting adjourned at 12:05 p.m.

T. E. Devine, Chair

E. R. Shipe, Vice-chair

SOYBEAN CROP GERMPLASM COMMITTEE MEMBERS

FEBRUARY 17, 1997

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SOYBEAN GENETICS COMMITTEE REPORT

The Soybean Genetics Committee met February 17, 1997 at the Sheraton Inn, St. Louis, MO in conjunction with the National Soybean Breeders' Workshop. Committee members attending the meeting were, G. R. Buss, B. Diers, B. R. Hedges, R. L. Nelson, J. H. Orf, R. G. Palmer, T. W. Pfeiffer, P. R. Arelli, and S. K. St. Martin. B. Diers and P. R. Arelli had been elected by mail ballot to serve a three-year term on the committee. Brian Diers was elected chair for the year ending February, 1998. Current Committee members and February expiration dates for their terms on the Committee are:

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Procedure

As in the past, manuscripts concerning qualitative genetic interpretation, gene symbols, and linkages should be sent to the Chairperson of the Soybean Genetics Committee for review. To facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol." Manuscripts will not be reviewed for style except as this influences the clarity of interpretation. Manuscripts will not be "peer reviewed" unless requested by the author. Authors may submit unpolished (but comprehensible) manuscripts for review, unless peer review is requested. This should reduce delays involved in publishing a paper.

2. Reviewers of manuscripts will be given a deadline of three weeks to return the reviewed manuscript to the Chairman (who will then return it to the author as soon as possible). If the reviewers have not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairman of the Soybean Genetics Committee.

Assignment/Approval of Gene Symbols

If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R. L. Nelson is Curator for all maturity groups. A form for this purpose is on page 24 of this volume.

Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretation does not depend upon symbols, in most cases.) The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is not necessary.

New Business

The committee recommended that the chair send a letter to the editors of several journals which publish soybean genetics papers to remind them that the committee approves gene symbols and requesting that they check about approval of gene symbols when reviewing a manuscript. Recommended journals are Crop Science divisions C1 and C7, Theoretical Applied Genetics, and Journal of Heredity.

The committee discussed establishing naming conventions and guidelines on the evidence necessary to assign gene symbols to QTL. Inquiries will be made to the genetics committees of other plant species as a starting point in this procedure.

[Summary of responses as of 3 March 1997 follows:

Arabidopsis does not have a naming procedure.

Maize names QTL as follows. In the maize data base, each reported QTL is given a unique name as follows: (q) + (3-5 letter abbreviation for the trait) + (a consecutive number). Thus, qgrwt2 is the second QTL for grain weight entered into the database; qplht64 is the 64th plant height QTL. When switching to a new experiment, population or environment, the sequential numbering is continued rather than starting over at 1.

Wheat names QTL as follows. The basic symbol for QTL should be 'Q'. The' Q' should be followed by a trait designator, a period, a laboratory designator, a hyphen, and the symbol for the chromosome in which the QTL is located. The designator should consist of no more than four and preferably three letters, the first of which is

capitalized. Different QTLs for the same trait that are identified in one chromosome should be assigned the same symbol except for the addition of a period and an Arabic numeral after the chromosome designation. All characters in the locus symbol should be italicized.

Rules of evidence from the different crops will be obtained.]

Suggestions on how to proceed in soybean are welcome; contact B. Diers.

Gene symbols/Linkages assigned March 1996 - February 1997.

Date	Authors	Trait	Gene Symbol/Linkage
17 Sept. 1996	Palmer Chen	Linkage Group 9	*Ap - Fr3* 4.5 to 10.2 cM (variable distance)
15 Jan 1997	Chen Palmer	Linkage Group (not assigned)	*k2 -y20* 3.0 cM

Guidelines on the Evidence Necessary for the Assignment of Gene Symbols

Researchers are strongly encouraged to send all gene symbols and genetic interpretations to the Soybean Genetics Committee for review prior to publication to avoid duplication and/or confusion. Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing.

The following is a set of guidelines prepared by the Soybean Genetics Committee and intended to help researchers undertaking genetic analysis of soybean traits. Of necessity, these procedures will often need to be modified by the researcher to fit the specific situation, but an application of these guidelines should aid in making the correct genetic interpretation.

1. A genetic hypothesis is made on the basis of classification of segregating progeny, usually the F_2 generation and here called the hypothesis generation.

2. A second generation with a pedigree trace to the first generation, is classified to confirm the proposed genetic hypothesis. This second generation may be progeny of the hypothesis generation (usually F_3) or progeny of a testcross (F_1 x recessive homozygote).

3. Traits that are strongly influenced by nongenetic factors require verification of the classification scheme by evaluation of the progeny from homozygous plants of the hypothesis generation. Testcross data are not suitable for this purpose.

4. For genes controlling a phenotypic expression similar to that of previously published genes, data must be obtained to test for uniqueness and allelism. This will usually require crossing a homozygous line carrying the newly identified gene with the original sources of the previously published genes. If appropriate allelism tests are not included in a manuscript, the committee will request such information from the researcher. Molecular linkages can also be used to demonstrate that the allelism test conducted is the only one needed.

5. Identification of cytoplasmic factors requires reciprocal crosses between parents differing in the trait of interest. Since these factors are transmitted through the cytoplasm, the trait is expected to be associated only with the maternal parent in the F_1 and succeeding generations. Maternal effects need to be distinguished from cytoplasmic effects by using reciprocal F_1 and F_2 data.

6. Conclusive evidence for cytoplasmic factors should rule out self pollinations and nongenetic factors associated with the maternal parent. Selecting parents for reciprocal crosses that differ in nuclear genetic traits (e.g., flower or pubescence color) in addition to possible cytoplasmic traits will provide evidence of cross- rather than self-pollinations by observed segregation for the nuclear genetic trait in succeeding generations.

7. Inheritance patterns in a hypothesis generation (F_2) and a confirming generation (F_3) are absolute requirements for differentiating between cytoplasmic factors and nuclear genetic traits.

8. Follow the guidelines (Rules for Genetic Symbols) published in the Soybean Genetics Newsletter to assign the symbol.

9. Submit the manuscript to the chair, Soybean Genetics Committee, for review of the genetic interpretation and approval of the gene symbol (see Soybean Genetics Newsletter for name and address). Please indicate in unequivocal terms your willingness to provide seed for allelism tests requested by researchers discovering genes with a similar phenotype. This does not

restrict your asking for a signature on a Material Transfer Agreement.

10. If the line in which the new gene occurs in not already in the USDA Germplasm Collection, you are strongly encouraged to send a seed sample of the line to the curator of the Genetic Type Collection for assignment of a T-number and maintenance of the seed (see the current Soybean Genetics Newsletter for name and address).

References

Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombinational values in heredity. Hilgardia 24:235-278.

Hanson, W. D. 1959. Minimum family size for the planning of genetic experiments. Agron. J. 51:711-715.

Immer, F. R. 1930. Formulae and tables for calculating linkage intensities. Genetics 15:81-98.

Immer, F. R. and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Mather, K. 1951. The measurement of linkage in heredity. Methuen and Co., Ltd. London. John Wiley and Sons, Inc. New York.

Sedcole, J. R. 1977. Number of plants necessary to recover a trait. Crop Sci. 17:667-668.

Rules for Genetic Symbols

I. Gene Symbols

a. Gene symbols will not be assigned to traits for which no inheritance data are presented.

b. A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below. Gene symbols may, however, be written on one line.

c. Genes that are allelic shall be symbolized with the same base letter(s) so that each genetic locus will be designated by a characteristic symbol base.

d. Gene pairs that govern the same phenotype (including duplicate, complementary or polymorphic genes) constitute multiple loci that should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: Y_1, Y_2, etc.) The numerals may be written on the same line as the base. (Example: *Y1*, *Y2*, etc.) This shall be the only use of numerals. Letter designations should not be used. The numeral 1 is automatically a part of the first reported gene symbol for each base but may be omitted only until the second symbol is assigned.

e. The first pair of alleles reported for a genetic locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: *Ab, ab*; *Ab* is allelic and dominant to *ab*.)

f. If two alleles are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion and the alleles may be differentiated by adding one or two uncapitalized letters as superscripts to the base. When more than two alleles exist for a locus, the additional alleles, or those symbolized subsequently to the pair first published, shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: *R*, r^m, *r*.) This shall be the only use of superscripts. The letters may be written on the same line as the base if preceded by a hyphen. (Example: *Rps1-b*, *Rps1-k*, and *Ap-a*, *Ap-b*, *Ap-c*.) The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The letters may be an abbreviation of a descriptive term.

   If independent mutations with the same or similar phenotype are identified at the same locus, until it is possible genetically to ascertain if they represent identical or separate alleles, the gene symbol should be followed by an identifying designation in parentheses. The identifying designation, which should **NOT** be in italics or underlined, can be the place where the mutation was found, the cultivar in which it was found, or any other relevant characteristic of the mutation. [Example: *ms1* (Tonica), or *ms1* (Ames 2).] This will ensure that possible subtle differences between the mutations, such as differences in DNA sequence, or unique pleiotropic side effects, are not overlooked by workers using those genes.

g. Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to

complete the base), and L for leaf shape.

h. The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clearcut. The decision for intermediate cases is at the discretion of the author, but should be in accordance with previous practices for the particular type of trait.

i. An underscore may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A_ represents both AA and Aa.)

j. A question mark may be used in place of a symbol when the locus or allele is unknown or doubtful. The name of the line in which the gene was identified should be included in the symbol, in parentheses. A hyphen preceding the question mark indicates an unknown allele at a known locus, the absence of a hyphen indicates an unknown locus. [Example: *Rps?* (Harosoy) an allele in Harosoy at an unknown locus or *Ap-?* (T160) an unknown allele in T160 at the *Ap* locus.]

k. Plus (+) symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

II. Isoenzyme Symbols and Protein Gene Symbols

The following set of guidelines is to be used when assigning gene symbols to isoenzyme variants. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybean.

a. A gene symbol (generally three letters) that indicates, as clearly as possible, the name of the enzyme should be used. [Example: Adh (alcohol dehydrogenase), Idh (isocitrate dehydrogenase).] The appropriate Enzyme Commission name and number should be used in the original article, when appropriate, to designate the specific enzyme activity being investigated.

b. The electrophoretic conditions used to characterize a locus or allele should be specified clearly and in sufficient detail to be repeated by others interested in using the locus in genetic studies. The electrophoretic mobility, or other properties of an allele, should be clearly described by the authors.

c. Publications should include a photograph and/or an interpretive zymogram that allows readers to visualize the variability described by the authors, as well as to ensure that subsequent work corresponds to the original study.

III. Probe detected loci

The following guidelines are to be used for assigning locus names to probe-detected (RFLP) loci. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybean.

a. Locus designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names. (Example: Iowa St. Univ., IaSU). The prefix shall not be necessary in publications

except as needed to distinguish numbers that would, without the prefix, be identical.

b. The prefix is followed by a string of letters and/or integers that identify the probe used to detect the locus by the originating laboratory. This probe-identifying string should be limited to no more than six characters. This string should be separated from the prefix by a hyphen. (Example: laSU-B317).

c. The probe-identifying string is followed by the restriction endonuclease used in the restriction digest of the soybean genomic DNA that was probed. The following abbreviations for restriction enzymes are recommended: EcoRI = I, EcoRV = V, HinddIII = H,DraI = D, RsaI = R, Bc1I = B, TaqI = T. (Example: laSU-B317I, laSU-B317T).

d. Duplicate loci detected by the same probe should be identified with the same letter and integer base differentiated by integers (1, 2, 3, 4, etc.) consecutively assigned in the order of publication. These numerals are to be separated from the base string by a hyphen. Example: laSU-B3171-1, laSU-B3171-2, etc.)

e. Upon publication of new RFLP loci, researchers are strongly encouraged to

1. make the probe identifying the locus/loci publicly available
2. make available the identity of the restriction endonuclease used to generate the mapped polymorphism
3. make available the identity of the genetic stock used to map the locus/loci
4. make available the molecular weights of the polymorphic fragments used to map the locus/loci

IV. Random Amplified Polymorphic DNA (RAPD) loci

The following guidelines are to be used in assigning names to loci that are mapped using RAPD technology. The system adopted here is that which is generally employed in other species in which RAPD loci have been mapped.

a. Locus designations should begin with a letter identifying the origin of the primer. (Example: Operon Technologies, O)

b. The origin of the primer is followed by the primer name. (Example: Primer number 14 from Operon Technologies kit A, OA14.)

c. The primer name is followed in subscript by the fragment size in base pairs of the amplified fragment that is being mapped. (Example: An 800 bp fragment amplified with Operon Technologies primer 14 from kit A, $OA14_{800}$).

V. Simple sequence repeat (SSR) or microsatellite loci

a. Locus designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names. (Example: Iowa St. Univ., laSU). The prefix shall not be necessary in publications except as needed to distinguish numbers that would, without the prefix, be identical.

b. The prefix should be followed by a string of letters that identify the core nucleotide repeat of the SSR followed by an identifying number. This string should be separated from the prefix by a hyphen and should not exceed eight characters. (Example: IaSU-at275, BARC-gata3412).

c. Upon publication of new SSR loci, researchers are strongly encouraged to

1. make available the oligonucleotide primer sequences required for amplification of the SSR

2. make available the identity of the genetic stock used to map the locus/loci.

VI. Linkage and Chromosome Symbols

a. Linkage groups and the corresponding chromosomes shall be designated with Arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.

b. Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc. The symbol Def shall denote deficiencies, Inv, inversions; and Tri, primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second Def 1b, etc. The first published inversion in chromosome 1 shall be designated with the Arabic numeral that corresponds to its respective linkage group number.

c. Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2 the second case, etc. The first published deficiency shall be symbolized as Def A, the Def B, etc. The first published inversion shall be symbolized as Inv A, and the second as Inv B, etc. The first published trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

VII. Cytoplasmic Factor Symbols

a. Cytoplasmic factors shall be designated with one or more letters prefixed by *cyt-*. (Example: *cyt-G* indicates the cytoplasmic factor for maternal green cotyledons, *cyt-Y* indicates that for maternal yellow cotyledons.)

b. Designations for specific cytoplasmic factors following *cyt-*, shall follow the same format as for gene symbols. Base letters chosen to indicate apparent relationships among traits will have common initial letters for all loci in a related group of traits. Initial letters will be consistent with initial letters designating nuclear gene traits. (Example: *cyt-G* green seed embryo, *cyt-Y2* yellow leaves, becoming yellowish green.)

VIII. Priority and Validity of Symbols

a. A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.

b. In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

IX. Rule changes

a. These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

APPLICATION FOR ENTRY INTO THE SOYBEAN GENETIC TYPE COLLECTION

Date: _____ T number (assigned by curator) _____

Submitted by: _____

Address: _____

_____

_____

_____

_____

_____

_____

Return to:
R.L. Nelson, curator
USDA Soybean Germplasm
Collection
Department of Agronomy
University of Illinois
1102 South Goodwin Avenue
Urbana, Illinois, 61801, U.S.A.

Strain Designation: _____

Genotype: _____

Phenotype: _____

_____

Parental Origin: _____

_____

_____

_____

When and where found
and by whom: _____

_____

_____

_____

Description

Maturity Group

Pubescence color _____

Seed coat luster and color _____

Flower color _____

Pod color _____

Stem termination _____

Pubescence type and density _____

Hilum color _____

other _____

_____

Special instructions for growing
or maintenance, if any: _____

_____

Literature References: _____

_____

_____

(List the reference(s) that first and best describe the discovery and inheritance of the trait. Please send relevant reprints to the curator.

Date seedlot received at Urbana: _____ Date T number assigned: _____

CÁTEDRA DE GENÉTICA Y MEJORAMIENTO
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La Plata, Buenos Aires, Argentina

José Bruno

HETEROSIS IN SOYBEAN

Introduction

The principles that satisfactory and completely explain heterosis are yet unknown. There are some hypothesis that intend to explain through different estimates realized. According to Moll et al. (1962), and Falconer (1981), the deviation of the progeny mean from the two parents is a function of the number of loci at which parents carry different alleles, and is proportional to the agronomic genetic diversity between the parents, assuming non-additive gene action is present in the species in some degree.

According to Wu et al. (1988), the definition of heterosis is as deviation of F_1 mean from the midparent (MP) mean where the offspring of a cross is better than the mean of its parents:

$H_{ij} = F_1 - MP$ Where: H_{ij} = Heterosis of cross i x j,

F_1 = Phenotype of the first filial (F_1) generation.

$MP = (P_i + P_j)/2$

Hybridization in soybean and studying heterosis for yield grain in progenies was achieved in different places. Weber et al. (1970) compared F_1 hybrids with midparent and best parent, released by five years, they found heterosis of 25.1% with midparent and 13.4% with best parent. Paschal and Wilcox (1975) evaluated 30 crosses and found a mean heterosis of 8% with midparent and 12 hybrids higher than best parent. Nelson and Bernard (1984) examined heterosis in F_1 using plots to obtain reliable yield estimates, they found a mean heterosis with midparent of 6.5% for 12 F_1 in two years. Gizlice et al. (1993) in F_2 estimated of average midparent F_2 heterosis was 9.3% with range of -1 to 19%. In this study the most was explained by the general combining ability of the strains, the same authors detected no heterosis for 100 seed weight and seed protein or oil concentration.

Materials and Methods

The experiment was conducted on campus of Agronomy Faculty La Plata University (34° 'LS, 57° 'W) during the optimal growing season 1995 and 1996. The first year hybrids were produced and in the second year F_1 evaluation was realized. In both years seed was not inoculated with *Bradyrhyzobium japonicum*. Furrow irrigation was applied as needed to avoid plant water deficit. The hybridization technique was the usual utilized for this species.

The genotypes used for hybridization were from International Centers (AVRDC and IITA) and National Institute (INTA).

AVRDC: G - 1920, G - 2039, G - 3901.

INTA: Rojas, Asgrow 3121, Carman, Bay, Conesa, Hodgson.

IITA: TGX - 1660-15F, TGX - 1674-3F, TGX - 1740-2F, TGX - 1740-6F.

The evaluation of F_1 was achieved by comparing 1 or 2 F_1 plants with the best plant of a group of 10 parent plants placed at both sides of F_1 plants. The observations were taken in stage of growing, development, and harvest for some agronomic characters.

Results and Discussion

The material obtained from crosses and its evaluation is shown in Table 1. These F_1 progenies were evaluated and showed different expression of midparent heterosis from nothing to twice the amount productivity of plant yield, it is the behavior of hybrid: G-3901 x TGX 1740-6F. Likewise the components of yield (plant height, number of branches, number of pods) showed the same relation. In this case, the results of evaluation are shown in Table 2. This cross produced a pod with 2 seed that originated 2 plants.

In the case of the single hybrid exhibited midparent heterosis for seed yield with a mean heterosis of 137%, higher than it was founded by Bernard (6.5%) and Gizlice (-1 to 19%).

In reference to yield component of a single hybrid were likewise high midparent heterosis for yield: plant height (42%), number of branches (269%), number of full pods (275%), length of pod (12%). 100 seed weight did not show midparent heterosis (-10%). This was possibly due to the great number of pods, that was caused by nutritional deficiency in seed formation, Gizlice et al. (1993) did not find this either.

With regard to precocity, the outstanding hybrid showed in days to flowering an inheritance of partial dominance and in relation of days to maturity did not show differences.

Within evolution of vegetative stages of a single hybrid and their comparison with midparents, it showed precocity in emergence (62%) and first nodes formation also a great number of nodes like female progenitor, but a great vigor in leaf size (length 8.6% and width 21%) allowing a hybrid of high photosynthetic capacity. This may play a role in determining potential seed yield.

The hybrid showed very good agronomic performance better than their progenitors, suggesting an ability of adaptation to environment of this place. Thus, maturity was uniform, the same as leaves dropping, and a good plant architecture, the stem was gross and branched, there was no sign of lodging, nor pod dehiscence. At harvest a great number of empty pods was observed (40%) higher than the midparents value. This is probably the result of nutritional deficiency, and the seed was medium size,

The heterosis shown is possibly explained by both the general combining ability between parents and genetic distance.

Acknowledgments

The author thanks Antonio G. Pisabarro for his criticisms and careful reading of the manuscript.

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Nelson, R. L., and R. L. Bernard. 1984. Production and performance of hybrid soybean. Crop Sci. 24-549-553.

Wu, J. S., and M. D. Li. 1988. An operational genetic theory of heterosis, with applications in animal and plant breeding. Proceedings 2nd International Conference on Quantitative Genetics.

1987. Sinauer Associates, Inc. Publishers. Sunderland, Massachusetts, pp:416-422.

Table 1. Different cross and evaluation with its parents.

Trait	Flower color	R1 days	R8 days	Seed yield g./plant	100 seed weight	Nodulation
F: Rojas	b	63	150	126.0	27.7	+
M: G-2039	p	87	178	26.9	13.7	
F_1	b	63	154	autof.		
F: Asgrow 3127	p	41	116	11.6	18.1	+
M: TGX-1740-6F	p	63	163	27.8	16.3	+
F_1	p	40	117	pos.autof.		
F: G-3901	b	82	175	52.1	23.5	+
M: TGX- 1740-6F	p	63	175	37.4	16.3	+
F_1: x 2Plants	p	77	174	106.2	17.2	-
F: Asgrow 3127	p	40	130	9.6	18.1	+
M:G3901	b	70	150	72.1	23.5	+
F_1	p	40	123	18.7		-
F: Carmen	b	73	163	4.0	14.4	-
M:Bay	p	62	152	14.8	24.4	+
F_1:X 2 Plants		71	158	17.1-	17.4	+/-
F: Asgrow3127	p	40	130	5.8	18.1	+
M: Conesa	p	62	162	17.0	17.6	
F:1	p	40	106			
F: G-3901	b	77	178	13.2	23.2	+
M: Asgrow 3127	p	36	130	8.0	18.1	+
F_1: avg. 3 Plants	b	71	170	autof.		
F: G-3901	b	77	183	32.2	23.5	+
M: Bay	p	62	154	24.4	24.3	+
F_1: avg. 2 Plants	b	76	autof.			
F: TGX- 1740-2F	p	62	179	10.8	16.0	+
M: G-3901	b	73	161	39.5	23.5	+
F_1	p	70	162	18.8	16.9	+
F: G-2039	p	87	178	68.0	13.7	-
M: Hodgson	b	70	154	25.0	21.4	-
F_1	p	87	166	8.3	17.8	-
F: TGX-1740-2F	p	63	162	19.0	16.0	+
M: G-1920	p	116	172	13.0.0	10.4	-
F_1	p	114	178	54.4	11.5	-
F: TGX 1660-15F	p	113	173	12.3	10.5	+
M: TGX 1681-3F	p	114	178	168.0	11.8	+
F_1	p	105	178	23.7	10.0	-

Table 2. Results of evaluation of cross showed high heterosis.

Trait	Female G-3901	Male TGX-1740-6F	F_1 Pl. 1	F_1 Pl.2	Midparent (%)	Best parent (%)
Emergence days	4	8	3	3	-50	-25
Cotyl.stage (days)	7	14	5	6		
V1 (days)	17	18	9	11		
V2 (days)	21	21	20	19		
V3 (days)	30	33	24	22		
V4 (days)	37	40	33	30		
V5 (days)	45	45	40	36		
V6 (days)	52	52	46	44		
V7 (days)	57		51	51		
V8 (days)	63		55	55		
V9 (days)	70		59	59		
V10 (days)	74		63	63		
V11 (days)	77		68	68		
R1 (days)	81	63	77	77	7	22
R2 (days)	88	73	87	87		
R3 (days)	100	88	116	116		
R4 (days)	116	122	124	124		
R5 (days)	124	130	131	131		
R6 (days)	140	145	143	143		
R7 (days)	156	158	155	155		
R8 (days)	178	175	174	174	-1.4	-0.6
Flower color	b	p	p	p		
Main stem length (cm)	52	97	107	105	42.3	9.3
1st pod height (cm)	6	17	16	18	48	
No. of branches	6	7	21	27	269	243
No. of full pods	92	136	476	379	275	214
No. of empty pods	91	215	272	157	40	-0.2
Grain yield/plant (gr)	52	37	120	92	137	104
Pod length (cm.)	40	33	42	40	12	2.5
Pod width (cm.)	11	9	9	8		
Number seed/pod	1.96	2.08	1.92	2.10		
Number locule/pod	2.24	2.37	2.26	2.67		
100 seed weight	21.5	17.3	18.1	16.5	-10.8	-19.5

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Prospecting soybean disease fungi in Northeast of Buenos Aires, Argentina

Introduction

Among several fungal diseases on soybean (*Glycine max* (L.) Merrill) in Argentina, the most important are: *Sclerotinia* stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary (Castello, 1969, Mitidieri, 1980a, and Mitidieri, 1981) and seed injury produced by several pathogenic fungi species: *Diaporthe/Phomopsis* complex, *Fusarium spp., Colletotrichum dematium var.* truncata, *Cercospora kikuchii and Alternaria* sp. (Barreto et al., 1981, Barreto, 1992, and Mitidieri, 1980b)

One of the objectives of the present research is to obtain variety resistance diseases. Prospection studies were developed in order to establish the most important for the area and to evaluate the behavior of introduced genotypes towards diseases.

Materials and Methods

The material used was the strains: TGX 1740-2F, TGX 1681-3F, TGX 1740-7F, TGX 1674-3F, TGX 1740-6F, TGX 1448-2E, TGX 1670-1F, and TGX 1660-15F provided by IITA (International Institute of Tropical Agriculture) and the strains, Asgrow 4422 and Asgrow 5308, provided by INTA (Instituto Nacional de Tecnologia Agropecuaria, Argentina).

Fungal pathogens of leaves and stems: Samples were taken from plot trial every twenty days from sowing until harvest. Fungi were cultivated on potato-dextrose agar using common phytopathological technics. They were identified by morpho-biometrical and cultural characteristics.

Fungal pathogens in seed: samples were kept under laboratory conditions (15-20°C) in paper bags.

Analysis was carried out on the fourth month after harvesting. The method applied was that of blotter test recommended by Neergaard (1974).

For the assessment, the following was determined: a) Germinative capacity (%) and b) contaminated seed (%), (including here, germinated and non-germinated seeds), showing the presence of mycelium and/or fruit bodies. Microorganisms were isolated and cultured in 2% APG for their identification.

Results and Discussion

Tables 1 and 2 show the species isolated from leaves, stems, and seeds.

The evaluation indicated that fungal pathogens on leaves and stems had a very low incidence, only appearing in some isolated plants.

The species found on seed were those common in soybean crops.

The low germinative capacity observed in all strains and the high percentage of seeds with *Alternaria* sp. and *Fusarium* sp. might be related to the environmental conditions during seed maturing period, mainly concerning the amount of rain and air humidity. These factors also determined a delay of harvest date.

The variation of percentage of seeds infected with *Phomopsis* sp. depended on soybean strains. The highest was for TGX 1740-2F and TGX 1740 - 7F.

These results demonstrate that in this area, the quality of soybean seed is mostly affected by fungal pathogens and environmental conditions in the late reproductive stages.

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Table 1. Fungi pathogens from leaves and stems

Strain	Pathogens
TGX 1740-2F	*Alternaria* sp.
TGX 1681-3F	*Alternaria* sp.
ASGROW 4422	*Alternaria* sp., *Diaporthe phaseolorum*
TGX 1740-7F	*Alternaria* sp.
TGX 1674-3F	*Alternaria* sp.
TGX 1740-7F	*Alternaria* sp.
TGX 1448-2E	*Alternaria* sp., *Phomopsis* sp.
ASGROW 5308	*Alternaria* sp.
TGX 1670-1F	*Alternaria* sp.
TGX 1660-15F	*Alternaria* sp., *Sclerotinia sclerotiorum*

Table 2. Fungi pathogens from seed.*

Strain	Pathogens (%)	Germinative capacity (%)
TGX 1740-2F	*Alt.* sp. 18 *Fus.* sp. 28 *Phom.* sp. 36 *Cerc. k.* 1	62
TGX 1681-3F	*Alt.* sp. 11 *Fus.* sp. 17 *Phom.* sp. 5 *Collet. d.* 1	58
ASGROW 4422	*Alt.* sp. 22 *Fus.* sp. 58 *Phom.* sp. 4	28
TGX 1740-7F	*Alt.* sp. 13 *Fus.* sp. 28 *Phom.* sp. 18	54
TGX 1740 6F	Alt. sp. 14 *Fus.* sp. 51 *Phom.* sp. 7	43
ASGROW 5308	*Alt.* Sp. 15 *Fus.* sp. 42 *Phom.* sp. 4 *Cerc. k.* 3	49

The fungi observed in the analyzed material were:

Alt. sp. = *Alternaria* sp.
Cerc. k. = *Cercospora kikuchii*
Collet. d.= *Colletotrichum dematium var. truncata*
Fus. sp. = *Fusarium* sp.
Phom. sp. = *Phomopsis sp.*

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A Basic Character of Responsiveness to Day Length of Wild Soybean (*G. soja)*

Abstract

When materials collected from throughout China were planted in Beijing in 1993, their growth periods showed a similar variation with those in their adapted places. The growth periods became longer as D1 values (accumulative day length hours from emergence to June 20th) were getting higher. However, differences between growing in their adapted places and growing in Beijing were noticed. Vegetative days in Beijing for materials from higher latitude were less than those in their original places as latitude was increasing. On the contrary, those vegetative days in Beijing for materials from lower latitudes were greater than those in their collected spots as latitude was decreasing. This phenomenon means that wild soybeans kept their basic requirements for day length formed in their adapted places, showing a highly genetic conservation. Geo-ecological isolations and permanent selection are the only reasons for the formation of diversity of wild soybean growth types.

Key words: wild soybean, day length, growth period

Growth period of wild soybean growing in natural environments is affected by synthetic geo-ecological factors. According to the previous studies, the most important factor is accumulative day length hours from emergence to bloom. Wild soybean can grow well if living in a suitable environment. However, when it is moved into a new environment, which is not good for its growing, it still can live if the plant can bear its new environment, especially its day length character, but abnormally grow, the plant could become small or flourishing, and seed could not set well. As well known, it is very important to choose a proper date to plant soybean in soybean production. Studying the basic character of responsiveness to day length of wild soybean would help us know how to determine a cultivar's planting date in order to produce high yield. The purpose of this research is to investigate changes of wild soybean's growth period when moved into a specific day length condition, and to explore the reasons leading to these changes.

Materials and Methods

120 accessions of wild soybean collected from the whole country were replanted in their nearest agricultural experiment stations 1982-1983 and planted in Chinese Academy of Agricultural Science, Beijing on April 30th, 1993, repeated two times. Emergence, bloom, and maturity were studied and recorded. Accumulated day length hours from emergence to June 20th (D1) in different collected spots also were investigated. A data base was set up by Foxbase and pictures were drawn by HG computer software.

Results and Discussion

When experimental materials from the whole country were replanted in Beijing, a variation that was similar with those planted in their local places was observed. Vegetative days increased with an increase in D1 values. However, there was a difference between growing in their adapted places and growing in Beijing (Figure 1). Vegetative days in Beijing for materials from higher latitude declined as D1 decreased (latitude increased). Blooms usually moved to an earlier time in Beijing than that in their original places. For example, vegetative days of material from Huma was 45, and became 28 in Beijing, advanced 17 days. On the contrary, blooms usually delayed for materials from lower latitude places, their vegetative days in Beijing were increasing as D1 increased (latitude decreased) (Table 1).

Wild soybean growing in a condition of lower D1 value in high latitude could bloom if the plant acquired its specific D1 value formed in its adapted place, for example in Huma. When the material was planted in Beijing, this D1 value could be obtained within 28 days instead of 45 days, so its bloom advanced and vegetative days became shorter. Material from the south, for example Wuhe, Anhui province, could receive the D1 value formed in its local place in 125 days in Beijing day length condition instead of 93 days. Its bloom delayed and vegetative days increased when it was planted in Beijing. From this analysis, we can see that wild soybean kept a primary requirement for day length formed in adapted places showing a highly genetic conservation.

Another important factor for flowering is photoperiod which changes regularly with change of latitude. If a plant could get its basic requirement for photoperiod formed in its adapted place, it would also flower. This statement will be demonstrated in a further study.

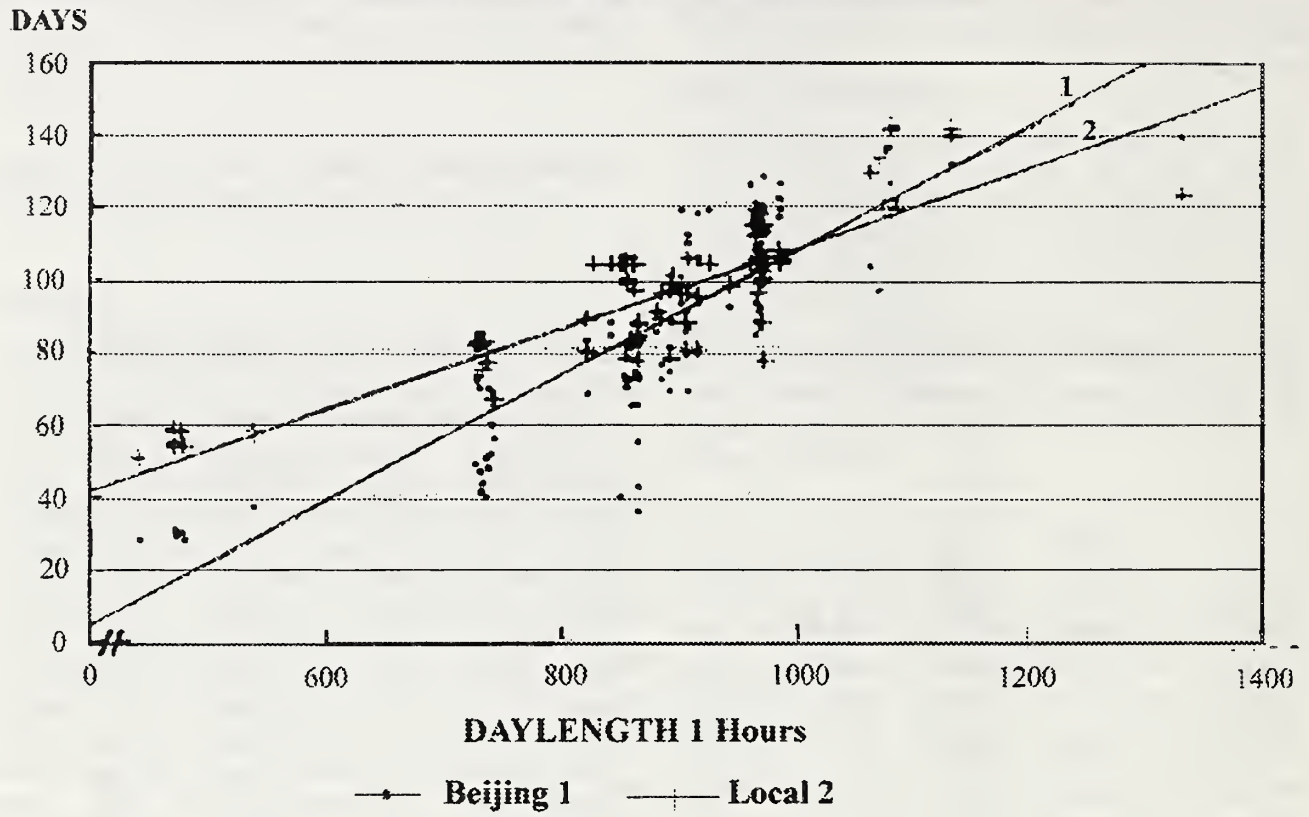
In conclusion, responsiveness to day length of wild soybean is genetically controlled. Geo-ecological isolation and permanent natural selection are the only reasons for formation of diversity of growth types of wild soybean (*G. soja*).

Table 1. Comparisons of growth periods of wild soybean growing in Beijing and in their adapted places.

Origin		Lat.*	Alt.* m.	D1* hur.	D2* hur.	Vegetative Beijing day	Days ori** day	Growth Beijing day	Days ori** day
Heilj***	Huma[1]	51°43'	178.2	441	1080	28	45	66	90
Heilj***	Nenj[2]	49°06'	243.0	477	1190	30	50	74	100
Heili***	Dedu	48°30'	273.2	472	1198	30	52	79	110
Heilj***	Harb[3]	45°45'	143.0	537	1225	55	57	107	120
Beijing		39°32'	40.0	862	1310	75	75	140	140
Shanx****	Dinx[4]	38°30'	960.4	883	1394	75	75	143	145
Shand*****	Jini[5]	35°13'	45.2	971	1498	102	80	170	160
Anhui	Wuhe	33°04'	21.0	960	1587	125	93	172	165
Hubei	Shnj[6]	31°26'	937.2	1081	1664	125	109	177	165

Note: *: adapted from Meterologic observation data on the earth's surface in China 1991-1995. Chinese Meteterological bureau
**: adapted from Inventory of Chinese wild soybean, 1990 Institute of Crop Germplasm Resources
***: Heilongjiang Province
1. Huma County
2. Nenjiang County
3. Harbin
****: Shanxi Province
4. Dingxiang County
*****: Shandong Province
5. Jining County
6: Shennongjia Region

FIGURE 1. VARIATION OF VEGETATIVE DAYS
Growing in Beijing and in Adapted Spots



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Comparison of Effectiveness Among Various Breeding Methods and Crosses on Resistance to Soybean Mosaic Virus (SMV)

Soybean Mosaic Virus (SMV) is one of the most prevalent viral diseases for soybean in China, as well as in east Asia, and even the world. The objectives of this study are to compare the effectiveness among various breeding methods and crosses on resistance to Soybean Mosaic Virus (SMV).

Materials and Methods

Six crosses of seven cultivars with differentiated resistance to SMV were made in 1982 (Table 1). Four selection methods (pedigree, bulk, picking-pod, synthesis) were used from generation F_2 to F_5, and 25 rows derived from each method were used to evaluate the resistant effectiveness at F_6. In 1988, 600 lines (6 x 4 x 25) were sown at Nanjing. All lines were divided into three grades by visual discrimination. Plants were classified into five grades according to disease index.

Results

1. Significant difference was expressed in effectiveness among crosses and selection methods on resistance to SMV (Tables 1 and 2).
2. The resistant effectiveness of the cross of two high resistant parents is the best, and next best is the cross "high resistant x medium resistant". The cross "high resistant x medium resistant" was better than the cross of two medium resistant parents (Table 1).
3. The rank of effectiveness among various selection methods was: pedigree > synthesis > bulk > picking-pod (Table 2).
4. The most number of resistant rows would be obtained from the cross R1 x R2 using the synthesis method, and the least number of resistant rows would be obtained from the cross R3 x r1 by the picking-pod method (Table 3).

Table 1. Effectiveness among various crosses on resistance to SMV

Cross	Type	H.r	M.r	M.s	Index	No. of resistant rows*	
R1 xR2	H. r x H.r	84	8	8	1.46	17.25a	A
R3 x r1	H.r x M.r	37	18	45	3.96	9.75c	BC
R1 x S1	H.r x M.s	41	20	39	3.52	14.50ab	AB
R2 x S1	H.r x M.s	71	8	21	3.37	11.25b	BC
r1 x r2	M.r x M.r	30	25	45	2.04	9.00c	BC
r1 x r3	M.r x M.r	21	23	56	3.53	8.50c	C

Notes: (1) R1, R2, R3, r1, r2, r3, and S1 represent AGS-9, Qihuang 1, Youbian 16, Suxi 5, Zhongdou 5, Xu 7319-3, and Jingtanbayuehuang, respectively. H.r: High resistant; M.r: Medium resistant; M.s: Medium susceptible.

(2) Different capital and small letters meant significant difference at 1% and 5% level. The same is true for the latter tables.

Table 2. Effectiveness among various selection methods on resistance to SMV.

Selection	H.r	M.r	M.s	Index	Resistant rows*	
Pedigree	94	18	38	1.68	15.33a	A
Synthesis	90	15	45	2.00	13.17a	A
Bulk	71	37	42	2.63	8.83b	AB
Picking-pod	59	22	69	5.37	8.50b	B

* Mean rows of six crosses.

Table 3. Number of resistant rows derived from crosses and methods

Selection	Cross						
Method	R1R2	R1S1	R2S1	R3r1	r1r2	r1r3	Average
Pedigree	21	20	13	14	13	11	15.33
Synthesis	22	16	12	10	8	11	13.17
Bulk	11	15	9	11	6	7	9.83
Picking-pod	15	7	11	4	9	5	8.50
Average	17.27	14.50	11.25	9.75	9.00	8.50	

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Detection of DNA Diversity of Wild Soybean (*Glycine soja*) in Natural Populations by New Procedure of RAPD and RFLP

Introduction

Compared with most plants, soybean as well as its wild relative, *Glycine soia,* showed low genetic diversity probably because of strict inbreeding in natural populations (Hu, Wang 1985; Bult, Kiang 1992; Yu, Kiang 1994). In this study, an improved procedure of randomly amplified polymorphic DNA (RAPD) and restriction fragment length polymorphisms (RFLPS) of amplified products were reported.

Materials and Methods

Total DNA was purified from fresh young leaves of wild soybean (*Glycine soia*) using the method of Stewart (1994). DNA amplification was conducted according to Williams (1990) except for the annealing temperature of 35°C instead of 36°C in a thermocycler manufactured by Chinese Military Academy of Medical Sciences (Beijing). Primers were purchased from Operon Inc. Electrophoretic separation and detection of amplified products (10μL) was performed as described by Williams (1990). At the same time, 1-3 μL of amplified products were separated on 6M urea 3.0 to 3.5% polyacrylamide gels, and detected by silver staining (Bassam, Caetano-Anolles 1993). Amplified products which did not show polymorphism on urea PAGE were incubated with restriction endonucleases: EcoRI, Hinf I, MspI, Sa1I, and TaqI to full digestion. Resulting restriction fragments were separated on 6M urea 6% polyacrylamide gel (PAG), and were detected by silver staining.

Results and Discussion

1. When the traditional method was used, RAPD of wild soybean only showed a few bands and showed low diversity within and among natural populations.

2. Urea PAGE separation of RAPD products cited above showed 20 to 60 silver- staining bands which number was much greater than the bands observed by agarose-ethidium bromide (EB) method. Experimental variability for both procedures was similar due to the high repeatability of PAGE.

3. Remove single EB-staining bands from a low melting agarose gel. After melting at 70° or 95°C, gel solutions directly loaded into slots of urea PAGs. Silver staining showed that each EB-staining band consisted of several fragments. Neighbor bands on an agarose gel shared some silver staining bands. Molecular heterogeneity of a single EB staining band separated on an agarose gel was comfirmed by gene cloning experiments using pUC19 as a vector.

4. Remove single silver-staining bands from PAG and amplified again. Gene cloning of re-amplified products by using vector pGEM-T (purchased from Promega) showed that the inserts shared the same molecular weight.

5. Using the improved RAPD procedure (above), the results of DNA diversity of a saline population of wild soybean were summarized in Table 1. Table 1 showed that the percentage of polymorphic loci varied from 0 to 65.4% with an average value of 34.3%.

6. After digestion of restriction endonuclease, some new fragments with molecular weight of approximately 50-500 bp increased from amplified products. As a result, the DNA profiles changed significantly. A higher concentration of PAGE (6%), therefore, became necessary to separate DNA fragments. The number of restriction fragments depended on the enzymes used. Generally, those enzymes which recognized six bases, such as EcoRI, produced fewer new bands, while HaeIII and HinfI recognized 4 bp and produced up to 10 new bands. No digestion has been found with TaqI. In most cases, enzyme digestion of RAPD, which previously shared the same profiles did not detect polymorphisms, i. e. same restriction profile shared. In a few cases, polymorphism occurred. For instance, when 7 amplified products derived from 7-plants in a population showed the same profile by using primer OPD05, a 362 bp fragment appeared in three plants after digestion. The other four plants

did not have the band. Most RFLPs of amplified products occurred in the HaeIII digestion.

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Table 1. RAPD of a population of wild soybean (*Glycine soja*)

Primer	Total Locus*	Monomorphic	Polymorphic	Percentage of Polymorphic
OPD01	39	25	14	35.9
OPD03	13	9	4	30.8
OPD04	16	6	10	62.5
OPD05	13	11	2	15.4
OPD07	15	12	3	20.0
OPD11	12	7	5	41.7
OPD15	24	21	3	12.5
OPD18	13	13	0	0.0
OPD20	17	7	10	58.8
OPJ04	26	9	17	65.4

* only the bands of lower molecular weight (less than 2.3kb) were scored. There were several bands with higher molecular weight in each lane, for example, when Primer OPJ04 was used, a total of 52 bands can be scored by visual observation. Those 26 bands with higher molecular weight were too crowded to distinguish their mobility differences.

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Effect of High Temperature and/or Drought Stress on the Activities of SOD and POD of Intact Leaves in Two Soybean (*G. max*) Cultivars

The changes in the activities of anti-oxidant enzymes during exposure of soybean to high/low temperature and water stress were observed recently (Deli et al., 1993 and Zuan et al., 1995). In this paper, we report the effect of high temperature and/or drought stress on the activities of SOD and POD in two different soybean cultivars.

Materials and Methods

Soybean seed ["Bainong 6#" (high drought-resistant) and "Jilin 27#" (less drought-resistant)] were grown on vermiculite supplemented with Hoagland solution in a plant growth chamber.

After 1-2 weeks, the seedlings were treated with the following: Treatment I: high temperature (HT), 45°C, 2-4h, Treatment II: drought (D), Hoagland solution with 4-6% PEG 6000 for 1 week, Treatment III: combination of HT and D.

The leaf-homogenates were centrifuged, and the supernatants were collected and used for the assays of activities of SOD and POD. SOD and POD activities were assayed spectrophotometrically, as described by Giannoplities and Ries, and Kochba, respectively.

Results and Discussion

1. In the control and stressed groups, higher or much higher SOD and POD activities in "Bainong 6#" leave extracts than those in "Jilin 27#" were seen. These results indicate that high SOD and POD activities in "Bainong 6#" leaves are linked with stress tolerance in plants that survive treatments likely to enhance the production of O_2- and H_2O_2.

2. In both "Bainong 6#" and "Jilin 27#", stress treatments of the leaves caused an increase in SOD and POD activities in comparison with the controls, respectively. With further increase in magnitude of high temperature and water stress in a certain range, SOD and POD activities showed a further increase (see the data in Treatment I and Treatment II, respectively).

3. It is noticeable that in "Jilin 27#" leave extracts, SOD activities in Treatment III were lower than those in Treatment II, and POD activities in Treatment III were lower than those in Treatment I and Treatment II. In contrast, in "Bainong 6#" leaves, SOD activities in Treatment III remained unchanged, and POD activities still increased significantly. These results indicate that the protective system composed of anti-oxidant enzymes of SOD and POD remained stable and endurable under severe stress conditions in "Bainong 6#" in comparison with those in "Jilin 27#".

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Table 1. Changes in SOD (Unit/mg. protein) and POD (OD_{470}/min. mg protein) activities in the leaf extracts of two soybean cultivars prepared from different treatments

Treatments	Jilin 27#		Bainong 6#	
	SOD acti.	POD acti.	SOD acti.	POD acti.
Control	211.23	403.39	221.17	670.66
Treatment I (HT)				
1. 45°C, 2h	231.15	453.37	332.28	756.38
2. 45°C, 4h	239.41	663.47	357.96	806.34
Treatment II (D)				
1. 4% PEG	284.88	588.58	319.81	804.52
2. 6% PEG	297.05	684.98	359.92	819.81
Treatment III (HT + D)				
1. 45°C, 2h + 4% PEG	264.17	454.78	349.05	1143.70
2. 45°C, 2h + 6% PEG	254.77	445.09	352.86	1218.42

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Efficient plant regeneration through somatic embryogenesis from germinated cotyledon of the soybean

Introduction

It has been reported that plants can be regenerated from the tissue of hypocotyl, cotyledon node, leaves, etc. of young embryos and even from a single cell and protoplast of the soybean. However, the frequencies are low and sometimes hardly repeatable, especially for protoplast culture and pollen culture. Researchers tried to set up a series of efficient plant regeneration procedures through somatic embryogenesis from the tissue of young embryos. Advantages of plant regeneration via somatic embryogenesis of the soybean were thought as follows: Firstly, the number of embryoids on the culture could be more than that of adventitious buds through organogenesis; secondly, embryoid formation might be more rapid than the process of organogenesis, and thirdly, the embryoid might already have a complete structure to develop directly into plantlet. Zhou et al. (1992) considered that immature organs were more suitable for plant regeneration than mature organs, and this was especially true for flowers. However, the explant source of immature embryo is restricted due to the short growing season. For a continuous availability of explant source, germinated seeds were the best choice if a high frequency of plant regeneration could be achieved. The objective of this study was to find an efficient and rapid plant regeneration procedure through somatic embryogenesis for using germinated cotyledon as the source of explants.

Materials and Methods

Experiment of genotypes and explant types

Eleven cultivars and breeding lines from Jiangsu, Henan, and Heilongjiang were used and explants were taken from the cotyledon and hypocotyl of 5-6 day old seedlings. The explants were cut into 2-3mm pieces. The induction medium used in the experiment was MS supplemented with 3% sucrose, 2.0mg/L 2,4-D, 1.0mg/L KT, and 1.0mg/L NAA (Medium C).

Experiment of induction medium and differentiation medium

The germinated cotyledon of Hongyin No. 1 was used as the source of explant. The induction media tested were as follows:

- Medium A, MS supplemented with 3% sucrose and 2.0mg/L 2,4-D
- Medium B, MS-macro and -micro nutrients supplemented with B5 vitamins, 3% sucrose, 0.2% casein hydrolysate, 2.0mg/L 2,4-D, 1.0mg/L BA, and 1.0mg/L IAA
- Medium C, MS supplemented with 3% sucrose, 2.0mg/L 2,4-D, 1.0mg/L KT, and 1.0mg/L NAA
- Medium D, MS supplemented with 20% potato extraction, and 2.0mg/L 2,4-D
- Medium E, MS supplemented with 3% sucrose and 0.25mg/L TDZ (Thidiazuron)

The induced calli from induction medium were transferred onto the differentiation medium. The differentiation media tested were as follows:

- Medium a, N6 basal medium supplemented with 2% sucrose
- Medium b, MS salts supplemented with B5 vitamins, 6% sucrose and 0.2% casein hydrolysate
- Medium c, Medium C but without auxins
- Medium d, MS supplemented with 4% sucrose, 0.5mg/L IAA and 0.3mg/L BA
- Medium e, MS supplemented with 3% sucrose, 0.5mg/L BA and 0.5mg/L KT

Culture conditions

The explants were cultured under 25±2°C, and exposed to 3000Lx daylight type fluorescent lamps, 16 hours daily. After 2-3 weeks culture, the induced calli were transferred onto the differentiation medium, or transferred onto the same medium as the original for sub-culture.

Results and Discussion

It was observed that the explant of hypocotyl easily formed callus, with a relatively rapid and large growth amount, and the explant was covered with calli after 18 days. The explant of cotyledon could also form callus efficiently, but the amount of calli was less and smaller than the former, and did not cover the whole explant, even after one month of culture.

Effects of genotypes and explant types on somatic embryogenesis

After 10-15 days culture of the explants from germinated cotyledon and hypocotyl on Medium C, there appeared some globular growth on the surface of the explant, especially near the cut site, which could be called the "globular stage"' of somatic embryo. Then those globular embryoids continued to develop through the cardiac, torpedo, and then to the mature stage, and some of them appeared to be leafy and axis-like. For all genotypes tested, calli could be induced and then somatic embryos could be differentiated, but there were significant differences of induction frequency and embryogenesis frequency among genotypes as well as between explant types (Table 1). The callus induction frequencies of the germinated cotyledon and hypocotyl from a same genotype were about the same for Hongyin No.1, Nannong73-935, and Heinong35, and could be up to 100%. For other genotypes, the callus frequencies of the cotyledon and hypocotyl differred greatly, such as Nannong86-4, in which the callus induction frequencies of hypocotyl was twice that of cotyledon and vise versa for Huaidou No. 2. As the embryogenesis frequency was concerned, there also existed differences among genotypes and between explant types. For Hongyin No. 1, Nannong73-935, NJ89 -1, Huaidou No. 2, and Heinong26, the frequencies of the cotyledon were higher than those of the hypocotyl, especially for Hongyin No. 1 and Nannong 73-935. The former were up to 33.3% and 30.1% much higher than 4.3% and 5.5% of the latter. For the others, there appeared no big difference between cotyledon and hypocotyl, except that for 18-69 and Heinong 35, the former appeared somewhat less than the latter.

Effect of medium components to somatic embryogenesis

After 4-6 days of culture, the cut surface of the explant turned brown and then swelled. There were obvious differences in the primary growth of callus and callus frequency among the induction media. The calli could be grouped into three types according to their morphological performance. The first type included those with many light yellow or green, compact, particle-like, and smooth structures, which usually could develop into embryoids. The second type was friable, translucent, somewhat sticky, and usually could not develop into an embryoid. The third type was composed of root primodia and had a strong rhizogenic ability. For convenience the three types of calli were denominated as "embryogenic callus", "non-embryogenic callus", and "rhizogenic callus", respectively.

The calli initiated on Medium E (Table 2) were fast growing, highly embryogenic, and had only a few rhizogenic areas. The highest frequency of embryogcnic callus (34.3%) was obtained on Medium E which differed considerably from the other four induction media. Medium A, B, and D induced embryogenic calli far less than Medium E. Medium C induced callus frequency was 23.6% which was 10.7% lower than Medium E. In addition, the number of embryoids increased well after subculture on Medium E. These results indicate that TDZ was more favorable to embryogenic callus differentiation in comparison to 2,4-D, BA, KT, and their combinations. The calli induced on Medium A, B, and D appeared dry, slow growing, and compact, with only a few globular or nodular structures. Therefore, the induction Medium A, B, and D were not favorable for somatic embryogenesis.

Somatic embryogenesis and its germination

Four weeks after swelling of the explants all the calli with embryoids were separated from the inoculum and transferred onto different regeneration media as indicated in Table 2. The small calli grew up to 3-5mm after 5-7 days, turned green, and then the embryoids started to germinate. These small calli usually could regenerate fully and produce multiple shoots. On the other hand, the big calli (larger than 10mm) usually could only develop roots and then became necrotic, but did not regenerate plantlets.

The highest frequency of calli with regenerated plantlets was 83% obtained from the induction-differentiation medium set C-e. However, the highest number of regenerated plantlets and that per callus were 14.6 and 2.4, obtained from the E-e set on which the frequency of regeneration from callus was 63%. In addition, only 4 of 164 regenerated plantlets were abnormal

on Medium e, which was much lower than the others.

Effects of genotypes and explant types

The results of this study showed that plant regeneration procedure using explants from germinated seeds was effective and efficient. This procedure could be used to substitute the use of explants from immature embryos due to its ease of attainability without constrains from growing season. Either germinated cotyledon or hypocotyl could be used as explant for plant regeneration. There existed genotype x explant type interaction. For some genotypes, cotyledon explant was better than hypocotyl explant, and vice versa. This might be due to the differences in physiological status and endogenous hormone levels between the two types of explants and among genotypes. However, germinated cotyledon was recommended due to its convenience of source and average high frequency of somatic embryogenesis.

Effects of hormones on somatic embryogenesis

It was reported that for somatic embryogenesis from immature embryo culture of the soybean a high concentration of 2,4-D (5-30m/L) in induction medium was required, but not cytokinin, which appeared to inhibit somatic embryo differentiation even at a very low concentration. This might be due to the existence of a high level of endogenous hormone in the soybean's immature embryo. However, for somatic embryogenesis from germinated cotyledon, a large amount of calli could be induced with relatively low concentration of 2,4-D, or the combination of 2,4-D, KT, and IAA, although the plant regeneration frequency of embryogenic callus varied greatly among the media, just as the results on Hongyin No. 1 indicated.

Thidiazuron (TDZ), a cotton defoliator, was reported to be of active function as a cytokinin in inducing embryogenic callus and improving bud differentiation frequency. In the present study, TDZ was also active and effective in inducing soybean callus, up to 100%, and in obtaining high frequency of embryogenic callus and plant regeneration.

Conclusion

Efficient plant regeneration was carried out by using five to six day old germinated hypocotyl and cotyledon as explants, and by adjusting the components of medium and hormones. The results showed that frequencies of embryogenic callus induced from germinated soybean cotyledon on MS media plus 0.25mg/L TDZ could be up to 34.3%. After the embryogenic callus was transferred onto MS medium with or without 0.5mg/L BA and 0.5mg/L KT, it turned green, and 63-83% of the embryoids germinated and produced shoots. The procedure was simple, highly repeatable and time saving, taking only one month and a half to obtain regenerated plantlets.

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Table 1. Frequencies of somatic embryogenesis of genotypes and explant types of soybean.

Genotype	Source of genotype	Number of explants		Number of calli		Frequency of induction(%)		Frequency of somatic embryogenesis(%)	
		cn	hy	cn	hy	cn	hy	cn	hy
Hongyin No. 1	Jiangsu	31	30	31	30	100.0	100.0	33.3	4.3
Nannong73-935	Jiangsu	23	28	23	28	100.0	100.0	30.1	5.5
Nannong88-48	Jiangsu	25	25	22	23	89.5	91.3	12.1	10.3
NJ89-1	Jiangsu	31	31	26	31	86.1	100.0	12.6	3.2
91-5	Jiangsu	21	27	19	25	86.8	92.4	19.1	22.6
18-69	Jiangsu	23	25	17	23	86.7	100.0	12.2	18.2
Nannong86-4	Jiangsu	21	19	9	17	41.7	87.6	14.8	12.2
Huaidou No. 2	Jiangsu	30	30	27	16	91.4	53.6	27.4	8.7
Yudou No. 2	Henan	17	25	10	17	49.2	66.3	11.7	13.0
Heinong26	Heilongjiang	25	27	23	27	92.2	100.0	24.2	11.7
Heinong35	Heilongjiang	31	30	31	30	100.0	100.0	16.7	24.8

Note: 1. hy = hypocotyl; cn = cotyledon.
2. All the figures are averages over four replications.

Table 2. Frequencies of callus induction and regeneration (Hongyin No. 1).

Item	Induction and differentiation medium				
	A	B	C	D	E
Frequency of callus induction(%)	93.2	93.4	98.0	96.7	100.0
in which, Embryogenic callus %	7.8	8.0	23.6	9.3	34.3
Non-embryogenic callus %	13.0	16.0	11.0	21.0	9.0
Rhizogenic callus %	79.2	76.0	65.4	69.7	56.7
	A-a	B-a	C-c	D-d	E-e
Number of embryogenic callus	48	55	60	68	60
Frequency of regeneration from callus	47.0	52.0	83.0	63.0	63.0
Number of regeneration plantlet	62	92	120	109	146
Number of abnormal plantlet	12	10	18	9	4
Regenerated plantlets per callus	1.3	1.7	2.0	1.6	2.4

Note: The embryogenic calli were those with more than two embryoid-like structures.

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Gametic Selection in a Cultivated/Semi-wild Soybean Cross Detected by Using RFLP Markers[2]

Segregation distortion of Mendelian inheritance has been reported for many genes and markers in a number of species (Lyttle, 1991; Grant, 1975). The most possible causes for distortions include: (1) competition among the gametes or zygotes, leading to different viability (Hormaza et al., 1990), and (2) errors of distinguishing individuals owing to phenotypes inappropriate to their genotypes. The segregation distortion of molecular markers is not affected by the latter effects and can directly reflect the results of gametic or zygotic selection. The phenomenon of gametic selection and its possible role has been studied and discussed in some species such as rice (Pham et al., 1990; Lin et al.,1992) and tomato (Nienhuis et al.,1992). Here we report the gametic selection present in soybean based on the analysis of the segregation of RFLP marker in a cross between cultivated and semi-wild soybean.

Materials and methods

An 84-plant F_2 population was originated from the cross of the cultivated accession 'Changnong4' with the semi-wild soybean 'Xinmin6'. Procedures of the RFLP analysis were described previously (Zhang et al.). The RFLP markers were provided kindly by Dr. R.C. Shoemaker, USDA-ARS and Iowa State University. For each RFLP marker locus, the observed ratio of segregations was tested against the expected Mendelian ratio (1:2:1 or 3:1) with the method of chi-square test. Two distorted segregation markers, the allele frequency (p,q) and the distribution of genotype frequencies according to (p^2:2pq:q^2) were tested to determine the occurrence of gametic or zygotic selection (Table 1) (Phain et al., 1990).

For dominant RFLP markers, heterozygotes can not be distinguished from homozygotes, so only the allelic frequency was estimated on the basis of the double recessive homozygote.

Table 1. Analysis for distorted segregation RFLP markers

χ^2 test		
p=q	p^2: 2pq : q^2	Selection Type
Significant	Non-significant	Gametic
Significant	Significant	Zygotic and Gametic
Non-significant	Significant	Zygotic

Results and Discussion

Of the 56 RFLP markers investigated in the F_2 population, 14 markers showed deviations from expected ratios by using the chi-square test. The frequency of the deviated markers was 25 percent, higher than that of another soybean F_2 population reported by Keim et al. (1990).

The allele frequency and the distribution of genotypes of the distorted segregation markers were summarized in Table 2. The allelic frequency of most markers showing distorted segregation deviated significantly from the expected ratio, but the distribution of genotype fitted the ratio from a random reassortment of gametes. According to the criterion described in Table 1, out of 11 codominant markers with the distorted segregation one was affected by zygotic selection, two were affected simultaneously by both the gametic and zygotic selection, and the remaining 8 were affected by gametic selection. In addition, the allele frequency of the 3 dominant RFLP markers with deviated segregation also differed significantly from expected ratio. This indicated that there was at least gametic effect on the disturbance of these marker's segregation. It was obvious that the gametic selection was the major cause for the forming of the distorted segregations. Moreover, the fact that seed fertility of the hybrid between the two parents studied was normal revealed that the gametic selection occured mainly in the male gametes.

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The direction of segregation distortion is apparently toward the cultivated parent by comparing the allele frequency of markers detecting skewed segregations. Except two markers, one with its allele frequency in favor of the semi-wild soybean parent and the other with its allele frequency fitting the ratio of 1:1, all the other markers show deviated segregation had their allelic frequency in favor of the cultivated parent. This shifting tendency could also be manifested by the frequency distribution of the cultivated parent allele in the F_2 population for all markers (Figure 1). This uni-direction favorness was not encountered in similar soybean F_2 populations used by Keim et al. (1990), but it existed in studies on other species (Lin et al., 1992; Nienhuis et al., 1992).

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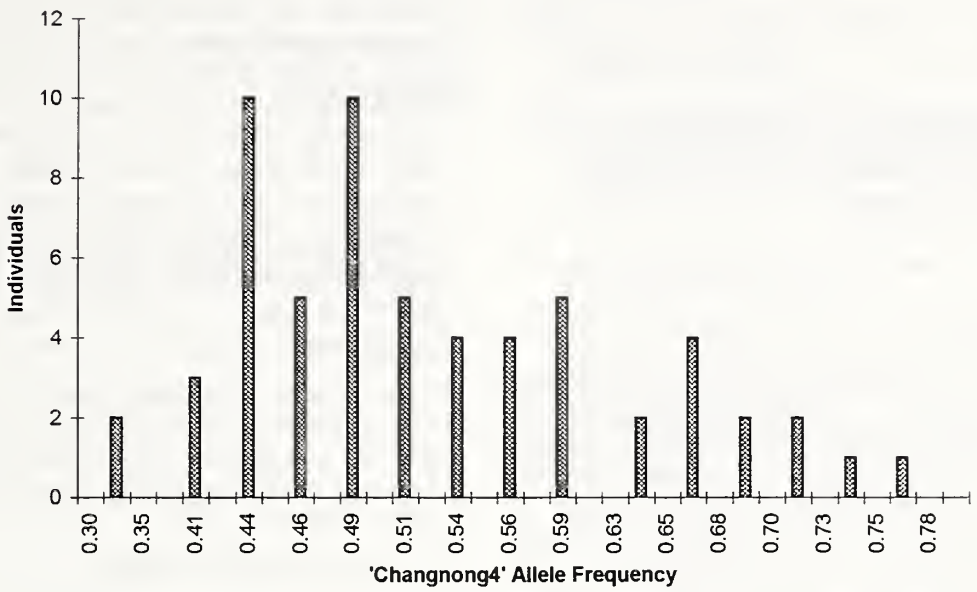
Table 2. Analysis of allele frequency for distorted segregation markers

RFLP Marker	Allele Frequency P1	Allele Frequency P2	χ^2 Allele Frequency Homogenity (P1=P2)	χ^2 F2 Distribution $p^2:2pq:q^2$
A18	0.638	0.362	10.512**	0.586
A398	0.613	0.387	7.253**	1.664
A112-a	0.675	0.325	19.600**	2.667
A885	0.655	0.345	10.571**	6.777**
A315-a	0.494	0.506	0.022	9.019**
K494	0.597	0.403	5.796*	2.508
A264	0.596	0.404	6.119*	3.287
A351	0.648	0.352	9.463**	1.231
A280	0.612	0.388	7.856**	1.415
A878	0.604	0.396	7.095**	0.649
A122	0.581	0.419	4.199*	6.711
A510-a	0.698	0.302	24.150**	
A112-c	0.724	0.276	31.711**	
A510-d	0.359	0.641	11.133**	

*and ** significantly at 0.05 and 0.01, respectively

Figure 1. Distribution of allele frequency of Changnong4 in Changnong4/Xinmin6 F2 population



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Genetic analysis of a null-allele for lipoxygenase-2.3 and Kunitz trypsin inhibitor in soybean

Abstract

Two crosses were made using 91D15-*titi* (F_5, Ludou No.4 x PI L83-4387) x Century-$lx_{2.3}lx_{2.3}$ and *ti*-15176 (F_5, Yudou No.8 x PI L81-4590) x Century-$lx_{2.3}lx_{2.3}$. F_2, F_3 progeny seeds were analyzed through NATIVE-PAGE and IEF-PAGE methods. A primarily genetic study was conducted and Chi-square test was carried out. An independent genetic relationship between *ti* and $lx_{2.3}$ is shown. A new type of soybean with null Kunitz trypsin inhibitor and lacking lipoxygenase L-2.3 (*titi* $lx_{2.3}lx_{2.3}$) was achieved. A further selection of agronomy and economical value is still going on.

Keywords: Soybean Lipoxygenase Kunitz trypsin inhibitor inheritance

Introduction

Soybean Kunitz trypsin inhibitor (KTI) is one of the most important antinutritional factors (Hymowitz, 1984 and Orf, 1979). Soybean lipoxygenase (Lox) is known to catalyze the oxidation of unsaturated fatty acids and polyunsaturated lipids containing cis-cis-1,4-pentadiene moieties such as linolenic acid. The oxidation products are associated with the development of grass-beany, bitter flavors and other problems in soybean products (Wolf, 1975). Heat treatment is often used in food processing for the elimination of both, but the processing is expensive and not entirely satisfactory of keeping the nutrients of the soybean protein. The genetic elimination is an alternative approach. American and Japanese scientists were successful in breeding cultivars of soybeans lacking KTI or Lox, and proved that above soybeans are not problems for the survival of the plants. (Hymowitz, 1984 and Kitamura, 1991)

This paper is about a new type of soybean with *titi* $lx_{2.3}lx_{2.3}$ lacking both KTI and $Lox_{2.3}$. This soybean may offer multiform of soybean for market to make the needs of different consumers in the future.

Materials and Methods

Soybean lines

91D15 (F_5, Ludou No.4 x PI L83-4387), *ti*-15176 (F_5, Yudou No.8 x PI L81-4590) were female parents with the absence of KTI by SBTI-A_2 analyzing. Century lines ($lx_{2.3}lx_{2.3}$) lacking $Lox_{2.3}$ were used as male parents obtained from USDA.

Cross seeds

Two crosses were made between 91D15 (KTI-less with normal Lox_8) and Century $lx_{2.3}$ (normal KTI but lacking $Lox_{2.3}$); between *ti*-15176 (KTI-less with normal Lox_8) and Century $lx_{2.3}$ (normal KTI but lacking $Lox_{2.3}$).

KTI and Lox, enzyme analyzing

F_2 seeds were analyzed by using Native-PAGE to distinguish the absence of KTI (Ding, 1990). After that *titi*-F_2 seeds were analyzed for identifying Lox types by IEF-PAGE (Liu, 1993).

Preliminary genetic analysis was carried out by Chi-square test.

Results and Discussion

F_2 seeds were obtained and analyzed to distinguish presence or absence of KTI by SBTI-A_2 test. The data of presence (A) or absence (a) SBTI-A_2 is shown in Table 1, the formula used was $X_c^2 = (|A-3a|-2)^2/3n$.

For all kinds of dominant gene seeds to the three recessive gene (*titi* $lx_{2.3}lx_{2.3}$) seeds, the observed ratio of presence (A) or absence (a) in the SBTI-A_2 protein and $Lox_{2.3}$ isozyme electrophoretic analysis was tested to see if the segregation fit the expected ratio of 63:1 (Table 2). The formula used was $X_c^2=(|A-63a|-32)^2/63n$.

From the above results, we consider the joint segregation of the presence or absence of the triple KTI and $Lox_{2.3}$ well fitted to the expected 63:1 ratio for three-gene inheritance. The triple lacking seeds (the rest half) were planted in the greenhouse or the field and showed normal growth, the seeds are not critical for the survival of soybean plants. A further selection of agronomical and economical value is still going on especially for disease resistance and pest defense, protein and oil content evaluation etc.

In this study a new type of soybean with null KTI and lacking $Lox_{2.3}$ was obtained. The F_2 seeds were analyzed for identifying both KTI and $Lox_{2.3}$, preliminary genetic Chi-square test was undergone. The triple lacking soybean seeds can germinate, grow, mature, and produce normal seeds both in the greenhouse and in the fields. Further selection for all important agronomic and economic value is going on.

Acknowledgments

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Table 1. Segregation of KTI in F_2 seeds

Cross	Genotype	Observed	Expected	Total(n)	X_C^2
91D15 X Century ($lx_{2.3}$)	*Ti*-(A)	52	54	72	0.017
	titi-(a)	20	18		
ti-15176 X Century ($lx_{2.3}$)	*Ti*-(A)	106	100	134	0.167
	titi-(a)	28	34		

Table 2. Segregation of KTI and Lox_2 in F_2 seeds

Cross	Genotype	Observed	Expected	Total(n)	X_C^2
91D15 X Century ($lx_{2.3}$)	*Ti*-$Lx_{2.3}$-(A)	71	70.875	72	0.127
	titi $lx_{2.3}lx_{2.3}$(a)	1	1.125		
ti-15176 X Century($lx_{2.3}$)	*Ti*-$Lx_{2.3}$-(A)	132	131.906	134	0.080
	titi $lx_{2.3}lx_{2.3}$(a)	2	2.094		

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Genetic engineering of male sterile and fertility restorer in soybean

In previous studies, attempts were made to obtain stable male sterility and fertility restorer systems through genetic engineering. By specific expression of a RNase gene in tapelal cells, male sterility was induced, and the male-sterile plants could be restored to male fertility by the specific suppression of cytotoxic RNase activity (Mariani et al., 1990).

We are attempting to create male sterility and its fertility restored system in soybean by manipulating genes that are essential for the production of viable pollen grains.

Materials and Methods

The soybean varieties used for genetic transformation are "Kefeng No.6" and "Youchu No.4".

The *Esherichia coli* strain DH55 was used as the host for cloning of plasmids, pBBN and pBBS. Two primers corresponding to the 5' regulatory region of anther-specific gene TA29 (Seurinck et al., 1990) were synthesized. The oligonucleotides were designed to contain Hind III and Nco I sites at the 5' end of each primer. Primers for the Rnase (barnase) gene and the RNase inhibitor (barstar) gene cloning were designed according to Hartley (1988). PCR reactions were run according to Sambrook et al. (1989). Plasmids were transferred to competent *A. tumefaciens*.

5-6 days after sowing the cotyledonary nodes of soybean variety were cut and infected overnight cultured and 10-20 fold diluted with *A. tumefaciens* cultures for 10 min. After drying on a filter paper, the infected explants were placed on differentiation medium (MS+1.25 mg/L BA +10mg/L Kanamycin), placed in the dark for 2-3 days, and then transferred to selective medium (MS+4 mg/L BA +10mg/L Kanamycin +500 Carpenicillin +20mg/L PPT) for shooting. The green shoots were transferred onto rooting medium (1/2MS+10mg/Lkanamycin +500mg/LCarpenicillin+20mg/LPPT+0.25mg/L NAA). Approximately 15-20 days later, the plantlets were transferred to the green house.

DNA was extracted from leaf tissue according to Dellaporta (1983). Total RNA was isolated from leaf, root, and anther tissue as described by Vires (1988). PCR assay, Southern hybridization, and Northern hybridization were carried out according to regular methods (Sambrook et al., 1989).

Results

We have cloned and sequenced the 5' regulatory region of an anther-specific gene TA29, an Rnase (barnase) gene and its specific inhibitor (barstar) gene. Cotyledonary nodes of variety "Kefeng No. 6" were infected with *A. tumefaciens*. Regenerated plants transformed with chimaeric barnase gene were male-sterile with shorter filaments, and shriveled anthers which were devoid of pollen grains. The abnormality of these transformed plants was proved to be associated with the destruction of the tapetal cell layers. Another variety "Youchu No.4" which could result in the best hybrid combination with "Kefeng No.6" was transformed with the barstar fusion gene. Southern hybridization showed that transformants contained bar and barstar hybridizing restriction fragments. Northern hybridization indicated that the barstar gene was regulated and expressed regularly in transformed plants. Our results suggests that by specific expression of barnase gene or barstar gene within anthers of transformed soybean, male sterility could be induced, and transgenic plants expressing the barstar gene which were expected to be the restorer of the male sterility could be obtained as well.

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Genetic Variability of Protein Content and its Selection Efficiency in the Early Generations of Soybean

Protein content is one of the important qualitative traits of soybean. To reveal the genetic property and the selection efficiency in early generations is of greater importance for soybean breeding.

Materials and Methods

Three soybean crosses, Suxi5 x Wujiangxiaomaodou (P1 x P3), Suxi5 x Qidongniutapian (P1 x P4), Suxie1 x AGS (P2 x P5), were made in 1987 at Nanjing, F_2, F_3, F_4 generation were obtained in 1988, 1989, 1990, respectively. In each year, 10 plants as a group of high, medium, low protein content, respectively, of each cross were selected from the previous three progeny.

A total of 93 blocks per cross with one row per block were studied in a randomized complete-block design with two replications. Each row was 2 meters with a 0.5 meter space between rows within a plot were grown in 1991 at Nanjing. Parents and F_1 progenies were one row and F_2, F_3, F_4 generations were 30 rows. 10 families of high, medium, low protein content, respectively, were sown for every F_2-F_4 progeny of each cross. 10 plants of each block were taken as a sample for the measurement of protein content.

Results

1. Different types of crosses showed different protein content in each generation. The rank of protein content of types of cross was:C1>C2>C3 (Table 1.
2. Additive effect was most important for protein content while that of dominant genes were also observed (Table 1).
3. Correlation coefficients between mid-parent and generations were greater than those between maternal or paternal parent and generations (Table 2).
4. The rank of variant coefficients of protein content was: Crosses>families within crosses>plants within family.
5. The heritability and genetic advances of protein content in soybean was generally high, which meant necessity and possibility of selecting plants with high protein content from early generation. Selection from F_2 generation would obtain more genetic gains for protein content than that from F_3, F_4 generation in three crosses (Table 3).
6. It showed effective to use the method of stratification for protein content in the F_2, F_3, and F_4 generations (Tables 4 and 5).

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Table 1. Means of protein content in parents and their crosses.

Cross	Type	Parent ♀	Parent ♂	Protein Content (%) ♀	♂	MP	F_2	F_3	F_4
C1	MXH	P1	P3	43.3	48.1	45.65a	46.88a	47.21a	47.53a
							1.23*	1.56*	1.88**
C2	MXH	P1	P4	43.3	46.9	45.10a	46.39a	47.01a	46.82a
							1.29*	1.01**	1.72**
C3	MXL	P2	P5	43.1	41.1	42.08a	42.54b	42.56b	42.96b
							0.46	0.48	0.88

Note: (1) H, M, L represent protein content range ≥ 44.0%, 43.01-44.01%, and ≤ 43.00%, respectively.
(2) MP: Mid-parent
(3) *, ** significant at 5% and 1% level, respectively, between mean and mid-parent value in three crosses.
(4) Different letters in same column mean significant difference at 5% level.

Table 2. Correlation coefficient of protein content among maternal and paternal parents, mid-parent, F_1, F_2, F_3, and F_4 generations.

Protein content (%)	Paternal parent	Mid-parent	F_1	F_2	F_3	F_4
Maternal parent	0.281	0.694	0.772	0.754	0.701	0.811
Paternal parent		0.702	0.777	0.784	0.717	0.792
Mid-parent			0.894	0.814	0.822	0.837
F_1				0.862	0.844	0.836
F_2					0.877	0.837
F_3						0.801

Table 3. Genetic parameters of protein content of F_2 and F_4 generations from three crosses.

Cross	CVg	CVp	H^2b	RGA	CVg	CVp	H^2b	RGA	CVg	CVp	H^2b	RGA
C1	4.20	3.86	84.1	7.27	3.45	3.17	84.57	6.54	3.30	2.39	52.64	3.51
C2	3.59	3.25	81.8	6.15	3.06	2.72	79.23	5.01	2.97	2.09	49.51	3.29
C3	4.46	3.28	54.16	4.98	4.42	3.12	54.20	4.72	2.73	1.98	52.69	2.95

Table 4. Analysis of variance of protein groups and lines within groups for protein content.

Generation	Resources		C1		C2		C3		
		DF	MS	F	MS	F	MS	F	F0.05
F_2	Group	3-1	8.94	50.14	7.19	7.82	3.46	2.97	3.33
	Lines within group	10-1	3.14	13.19	10.14	5.21	1.90	1.53	1.85
F_3	Group	3-1	10.17	48.94	6.14	5.43	4.14	2.71	3.33
	Lines within group	10-1	4.12	12.14	3.93	5.14	1.92	1.35	1.85
F_4	Group	3-1	8.45	47.32	5.24	3.19	3.84	2.73	3.33
	Lines within group	10-1	3.29	10.13	10.26	4.26	1.73	1.43	1.85

Table 5. The means and variation range of the protein groups for protein in the F_2 - F_4 generations of three crosses of soybean.

Cross	Protein	F_2		F_3		F_4	
	Group	Mean	Range	Mean	Range	Mean	Range
C1	H	46.9a	45.2-48.7	46.8a	43.4-50.1	46.6a	41.4-51.0
	M	45.3b	44.1-46.0	44.4b	39.2-47.9	44.9b	39.7-49.6
	L	43.4b	42.1-43.9	44.7b	40.9-48.4	45.8b	44.4-47.7
C2	H	45.7a	45.0-46.7	47.7a	43.2-50.8	47.6a	44.1-54.6
	M	43.8a	43.2-44.8	46.5b	43.6-48.5	46.5b	44.0-54.4
	L	41.9b	40.1-42.9	46.0b	43.2-48.5	46.6b	44.2-49.6
C3	H	43.8a	44.5-48.0	42.8a	37.1-46.6	43.0a	40.3-46.9
	M	42.7a	42.0-43.7	42.9a	39.7-45.3	43.3a	39.4-45.9
	L	40.7a	40.2-41.5	41.9a	36.5-44.6	43.3a	39.1-46.4

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On the natural selection effect in soybeans due to the winter and spring nursery conditions of Hainan Island

Introduction

Natural selection is an important vector to cause the genetic variation of the population structure. Luedders (1978) reported that there existed negative natural selection to early mature soybean. Winter nursery has been used extensively as an important breeding procedure to add one or two generations during winter to spring in tropical areas. Tian et al. (1978 & 1988) found the soybean performed different in plant height and yield when it planted and selected in Northeast China and Hainan Island (tropical area). The present paper was to exam how was the natural selection effect in soybean due to the winter and spring nursery conditions of Hainan Island.

Materials and Methods

The F_6 populations of 12 crosses from a 4X3 NC 11 design were planted in 1984 in Jiangpu Agricultural Experiment Station of Nanjing Agricultural University. The four female parents from southern China were Nannong133-3 (*TTwwdt1dt1Dt2Dt2*), Nannong1138-2 (*TTWWdt1dt1Dt2Dt2*), Suxie1 Hao (*ttww dt1dt1Dt2Dt2*), and Nannong493-1 (*ttww dt1dt1Dt2Dt2*). The three male parents from Huang-Huai area were Xuzhou 424 (*ttWWDt1Dt1dt2dt2*), Yuejin5 Hao (*ttwwdt1dt1Dt2Dt2* or *ttwwdt1dt1dt2dt2*), and Xudou4 Hao (*ttWWDt1Dt1dt2dt2*). The F_7 populations were planted in winter in Hainan, and two sets of seeds for each cross were obtained through picking one pod per plant, which were thought to be not influenced by natural selection, and the remaining pods were harvested together for each cross as a population after natural selection due to winter conditions. In the next gcneration (F_8) in spring in Hainan Island, one set of each cross was grown for picking up one pod per plant to obtain a population without natural selection, and another set of each cross was grown and harvested as a population with one time (winter) of natural selection. The mixed population obtained in 1984 winter was grown and still mixed in 1985 spring in Hainan as a population with twice (winter and spring) natural selection. The experiment was carried out in F_9 during the summer of 1985 in Jiangpu, Nanjing in a split-plot design with 2 replications, and with cross main plot and the different natural selection times sub-plot. The tested population of each cross was designated as R_0, R_1, and R_2 to represent those receiving 0, 1, and 2 times of natural selection. There were about 200 plants in each sub-plot. The frequencies of stem termination, pubescence color, pubescence type, flower color, plant height, number of nodes on main stem, 100-seed weight, and maturity date (days from planting to harvest) were investigated, and the data were analyzed by using the appropriate analysis of variance procedure. (Ma, 1982a & 1982b)

Results and Discussion

1. Performance of the five dominant genes of morphological traits

The frequencies of five dominant genes of morphological traits (determinate, semideterminate, purple flower, tawny pubescence, and thick pubescence) of the R_0, R_1, and R_2 population of 12 crosses are listed in Table 1. There showed no obvious tendency of traits along with the number of natural selection times. Especially, the frequencies of purple flower and tawny pubescence were relatively stable and fluctuated just around some value. For example, in the crosses Nannong493-1 x Xuzhou424 and Nannong133-3 x Xudou 4, the frequencies of flower color varied around 0.5, which indicated that these gene frequencies might have been fixed. The frequencies of Dt1, and Dt2, fluctuated among R_0, R_1, and R_2 in some crosses such as Suxie1 Hao x Xudou4 and Nannong493-1 x Xudou 4, but no obvious tendency was observed from the whole set of crosses. This kind of fluctuation might be due to both random effect and unsuitable judgment in distinguishing the phenotypes of the two genes.

A low proportion of off-type was found in the crosses which had the same genotype of parents, and these should not exist in the allelic genotype in the tested populations, such as those for flower color and pubescence color. However, off-type frequency was also stable enough, with only a very small fluctuation, which also indicated the population was not influenced with natural selection.

2. Performance of four quantitative traits

No obvious change of the quantitative traits plant height, 100-seed weight, and maturity among R_0, R_1, and R_2 was found in the 12 crosses (Table 2) except for number of nodes on main stem. The results of analysis of variance (Table 3) showed that there existed very significant differences in each of the four traits among the 12 crosses, and only significant difference in number of nodes on main stem among R_0, R_1, and R_2. There was no significant interaction of cross x population found in the four traits. Accordingly, the significant main effect of number of nodes on main stem among R_0, R_1, and R_2 was of practical meaning, and indicated the existence of natural selection effect, especially due to the second time (spring) natural selection. All other fluctuation among the three treatments might be due to some random factors.

Conclusion

There was no significant effect of natural selection on some major morphological traits and quantitative traits after one time (or winter) natural selection in Hainan Island. This was also true for twice natural selection from winter to spring in Hainan Island except that the number of nodes on main stem decreased due to the second or spring natural selection in Hainan Island.

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Table 1. The frequency of five dominant genes in the selection treatment populations of 12 crosses

CROSS	Dt_2			Dt_1			W			T			P_2		
	R_0	R_1	R_2	R_0	R_1	R_2	R_0	R_1	R_2	R_0	R_1	R_2	R_0	R_1	R_2
Nannong133-3 x Xuzhou424	0.27	0.27	0.31	0.23	0.23	0.40	0.43	0.33	0.37	0.36	0.36	0.37	0.56	0.61	0.72
Nannong1138-2 x Xuzhou424	0.47	0.41	0.51	0.09	0.10	0.07	1.00	0.99	0.99	0.61	0.51	0.60	0.18	0.10	0.13
Suxie 1 Hao x Xuzhou424	0.44	0.49	0.30	0.20	0.25	0.12	0.62	0.58	0.48	0.00	0.03	0.04	0.61	0.53	0.45
Nannong493-1 x Xuzhou424	0.42	0.35	0.33	0.29	0.32	0.27	0.49	0.50	0.48	0.01	0.01	0.03	0.55	0.47	0.56
Nannong133-3 x Xudou 4	0.44	0.37	0.44	0.15	0.22	0.38	0.53	0.41	0.44	0.25	0.24	0.26	0.61	0.58	0.66
Nannong1138-2 x Xudou 4	0.34	0.35	0.12	0.14	0.22	0.34	0.98	0.78	0.84	0.65	0.38	0.55	0.34	0.37	0.40
Suxie 1 Hao x Xudou 4	0.72	0.54	0.52	0.11	0.23	0.21	0.47	0.46	0.48	0.01	0.03	0.05	0.75	0.68	0.73
Nannong493-1 x Xudou 4	0.28	0.52	0.30	0.58	0.27	0.59	0.37	0.35	0.36	0.01	0.02	0.04	0.70	0.17	0.75
Nannong133-3 x Yuejin 5	0.17	0.14	0.27	0.03	0.06	0.05	0.08	0.13	0.06	0.82	0.80	0.79	0.65	0.65	0.87
Nannong1138-2 x Yuejin 5	0.04	0.01	0.02	0.03	0.00	0.02	0.53	0.39	0.47	0.39	0.42	0.29	0.34	0.49	0.42
Suxie 1 Hao x Yuejin 5	0.12	0.01	0.09	0.00	0.00	0.01	0.04	0.01	0.03	0.01	0.00	0.01	0.43	0.37	0.37
Nannong493-1 x Yuejin 5	0.00	0.04	0.12	0.05	0.10	0.01	0.04	0.02	0.02	0.01	0.00	0.01	0.57	0.73	0.59
Average	0.31	0.29	0.28	0.16	0.17	0.21	0.47	0.41	0.42	0.26	0.23	0.25	0.52	0.48	0.55

Table 2. Performance of quantitative traits in the selection treatment populations of 12 crosses

CROSS	Plant height (cm)			No. of node			100-seed Weight(g)			Maturity (d)		
	R_0	R_1	R_2	R_0	R_1	R_2	R_0	R_1	R_2	R_0	R_1	R_2
Nannong133-3 X Xuzhou424	71.4	56.8	67.9	19.6	16.1	16.3	17.3	15.8	16.4	121	117	118
Nannong1138-2 X Xuzhou424	67.7	60.5	61.1	17.1	17.5	15.7	18.1	18.2	18.4	120	120	120
Suxie 1 Hao X Xuzhou424	70.7	67.4	51.7	17.4	17.4	14.8	17.1	17.4	16.8	122	122	122
Nannong493-1 X Xuzhou424	64.1	63.5	61.6	17.0	17.1	16.9	17.9	19.0	17.9	131	127	123
Nannong133-3 X Xudou 4	73.4	62.6	79.5	17.8	19.2	18.3	15.3	15.5	15.2	128	121	128
Nannong1138-2 X Xudou 4	60.2	63.4	60.7	16.4	17.0	15.1	18.4	18.0	19.2	119	120	119
Suxie 1 Hao X Xudou 4	72.7	65.2	68.0	15.0	16.1	16.1	17.9	17.8	17.8	124	123	123
Nannong493-1 X Xudou 4	73.2	69.4	68.4	20.1	19.9	19.6	16.9	17.5	18.9	126	125	125
Nannong133-3 X Yuejin 5	57.5	45.3	62.3	15.0	15.1	14.8	16.1	17.1	15.4	122	122	122
Nannong1138-2 X Yuejin 5	43.7	48.0	49.0	13.6	13.7	12.7	16.8	17.3	17.6	117	116	116
Suxie 1 Hao X Yuejin 5	50.5	55.3	49.1	15.3	15.3	13.9	19.9	20.5	20.5	123	123	123
Nannong493-1 X Yuejin 5	41.3	56.8	45.9	13.6	14.7	13.8	19.9	19.7	16.5	136	136	132
Average	62.6	59.5	60.4	16.5	16.6	15.6	17.6	17.8	17.5	124	123	123

Table 3. Analyses of variance of four qualitative traits

Sources of Variability	Degree of freedom	Mean square and its significance			
		Plant height	No. of nodes	100-seed Weight	Maturity
Replication	1	445.55*	0.32	0.01	98.1
Cross	11	447.40**	20.41**	10.59**	149.44**
Error (A)	11	75.24	0.55	1.03	
Major block	23				
Populations	2	58.64	6.26**	0.49	9.40
Cross X Population	22	85.61	1.29	1.17	4.84
Error (B)	24	44.04	0.69	0.69	6.11
Total	71				

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The performance and allelism study of the new male sterile mutant NJ89-1 of the soybean

A male sterile mutant NJ89-1, arising spontaneously from a F_6 plant of the cross [(NanNong 1138-2 x NanNong 493-1) F_3-1-9-3-2 x YouBian 30] of the soybean, was found by Ma et al., 1993) at Jiangpu Experimental Station, Nanjing Agricultural University. Artificial pollination experiments revealed that the female fertility of sterile plants of NJ89-1 was not abnormal. The pollen germination experiments showed that the male sterility of NJ89-1 was pretty high and stable with a pollen germination percentage less than 0.75%. Then NJ89-1 was a complete male-sterile mutant. The genetic data led to the conclusion that the male sterility of NJ89-1 was controlled by a single recessive nuclear gene. In soybeans, eleven complete nuclear male sterile mutants, including male-sterile female-fertile mutants *ms1, ms2, ms3, ms4, ms5, ms6,* and synaptic mutants *st, st2, st3, st4, st5*, have been reported so far. Is NJ89-1 different from the reported eleven (*ms1-ms6, st, st2-st5*) complete male sterile mutants, or in other words, is NJ89-1 a new complete nuclear male sterile mutant? The present paper is to report the results of the performance and a series of allelism study of NJ89-1.

Materials and Methods

Progeny test and chi-square criterion were used to study the inheritance of NJ89-1 sterility. NJ89-1 male-sterile plants were originally obtained from a natural pollinated and sterility-segregating population. In 1993 seeds from NJ89-1 sterile plants were planted in fields. At maturity only fertile plants (designated as S_0) were individually harvested. In 1994 $S_{0,1}$ families were derived from S_0 fertile plants. In 1995 $S_{1,2}$ families were obtained from $S_{0,1}$ fertile plants. The ratios of fertile to sterile plants in segregating $S_{0,1}$ and $S_{1,2}$ families, and the frequency of segregating and nonsegregating $S_{1,2}$ families were observed.

Allelism tests of the NJ89-1 sterility with five designated male sterile materials, *ms1* (T266), *ms2* (T259), *ms3* (T273), *ms4* (T274), and *ms5* (T277), were conducted through making crosses between the heterozygotes of NJ89-1 and the five designated male sterile materials. If two lines were allelic with regard to their sterility, one out of four S_0 (S_0 designating the hybrids from a cross between two heterozygotes) plants would be sterile since all the male sterile genes were recessive, and in $S_{0,1}$ both nonsegregating families and segregating families with 3 fertile:1 sterile plant ratio would be observed. Otherwise, if different genes controlled the sterility in the two lines no sterile plants would be observed in S_0, and moreover, the $S_{0,1}$ would include nonsegregating families, families segregating in 3 fertile:1 sterile plant, and families segregating 9 fertile:7 sterile plants.

In addition to the allelism tests, general squash preparations, paraffin sections, transmission electron microscope (TEM), and scanning electron microscope (SEM) techniques were used to study the cytomorphological features of anther and pollen development of both NJ89-1 sterile and fertile plants. Then the results of NJ89-1 mutant were compared with those of the known mutants reported in the literature.

Results

In 1994 seventeen $S_{0,1}$ families were obtained. These families were all segregating fertile and sterile plants. The ratio of fertile to sterile plants was 742:239 which fitted the expected ratio of 3:1 (Table 1). Chi-square tests for homogeneity indicated that all families were drawn from the same population (Table 1). In 1995 181 $S_{1,2}$ families were derived from $S_{0,1}$ fertile plants. The frequency of segregating and nonsegregating families was 114:67 which fitted the expected frequency of 2:1 (Table 2). Among the 114 segregating families 3994 plants were fertile and 1276 were sterile which fitted the expected ratio of 3:1 (Table 1). Again all families appeared to be drawn from the same population (Table 1). These results supported the conclusion by Ma et al. (1993) that the NJ89-1 sterility was conditioned by a single recessive gene.

No sterile F_1 plant was observed in any of the five different allelism testcrosses including their reciprocal ones, which indicated the new mutant gene was not allelic to *ms1* through *ms5* (Table 3). Although the number of S_0 plants for each testcross was not large enough, the probability of zero sterile plants under the hypothesis of allelic existence would

be $(1/4)^n$ (n being the number of tested S_0 plants), which was very small for those testcrosses with *ms2, ms3, ms4*, and even *ms1*. However, that kind of probability for the testcross with *ms5* was $(1/4)^3$ = 1/64, not small enough, accordingly further facts were needed for demonstrating the non-allelic hypothesis between NJ89-1 and *ms5*. Unfortunately the $S_{0,1}$ data were not obtained and could not be used here.

Cytomorphological studies on anther and pollen development showed that NJ89-1 mutant was different from *ms1-ms6* in a large number of aspects such as abortion stage, meiosis, pollen wall, and anther wall etc. For example: (1) Abortion stage. Abortion occurred earliest at prophase I in NJ89-1, while abortion happened soon after telophase II in *ms1, ms4, and ms6*, shortly after tetrad stage in *ms2* and *ms3*. (2) Meiosis. Many meiotic abnormalities, including univalent, chromosome reduction, chromosome lagging, chromosome bridge, chromosome dividing into groups, unequal distribution, and micronuclei etc., were observed in NJ89-1. These abnormalities led to produce abnormal dyads, triads, tetrads, and polyads. Eventually sterile pollen grains varied greatly in size, were shrunken, and unvital. Failure of cytokinesis after telophase II and formation of coenocytic (quadrinucleate) microspores (CMs) were found in *ms1* and *ms4*. But the fates of the CMs were different between *ms1* and *ms4*. In *ms4* the CMs either degenerated soon or underwent some irregular divisions forming various kinds of 1- to 4-celled aggregates that degenerated sooner or later. While in *ms1* the CMs never exhibited the same type of division as observed in *ms4*, degenerated after formation. In *ms6* the process of microsporogenesis did not progress beyond telophase II, proper distribution of chromosomes led to normal telophase II however the tetrad stage was not observed and cells with degenerating nuclei were observed. In *ms2* and *ms3* meiosis was normal and tetrads were formed, but shortly after the tetrad stage microspore degeneration commenced. (3) Pollen. The NJ89-1 sterile plants produced pollen grains varying greatly in size, shrunken, and unvital. The *ms1* produced large degenerated coenocytic microspores. The presence of cellular aggregates instead of individualized pollen grains seems to be a unique feature of *ms4*. The *ms2*, *ms3*, and *ms6* had degenerating microspore tissue. The *ms5* had small and shriveled pollen grains. (4) Anther and flower. NJ89-1 and *ms1* had normal anther. The *ms2* produced reduced, shrunken, collapsed, and indehiscent anthers, *ms3* produced distorted and shrunken anthers, *ms4* produced slightly small, whitish instead of yellow, shriveled, and incompletely dehiscent anthers, *ms5* produced dark and shrunken anthers, and *ms6* produced reduced and shrunken anthers. NJ89-1 *ms1, ms2, ms3, ms4*, and *ms5* had normal flowers, and *ms6* had smaller flowers. NJ89-1 mutant displayed meiotic abnormalities such as univalent etc. of synaptic mutants. In soybeans, five synaptic mutants (*st, st2-st5*) have been reported so far. But NJ89-1 mutant differed greatly from st and st2-st5 mutants in the female development of sterile plants, namely, the female of NJ89-1 sterile plants was fertile while those of st and st2-st5 sterile plants were strongly impaired.

In summary, NJ89-1 was demonstrated as a new male-sterile mutant with abnormal chromosome synapsis, which performed similar with *ms* genes in the male-sterile and female-fertile feature, and different from *ms* genes in the abnormality of synapsis, and on the other hand performed different from *st* genes in the female-fertile feature, and somewhat similar in the abnormality of synapsis of male meiosis. In this case, it is preferred to classify it as a male-sterile mutant, and to designate it as a *ms* gene.

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Table 1. Ratio of fertile to sterile plants in segregating $S_{0,1}$ and $S_{1,2}$ families of NJ89-1

	$S_{0,1}$ families					$S_{1,2}$ families				
	Fertile plants	Sterile plants	df	x^2(3:1)	P	Fertile plants	Sterile plants	df	x^2(3:1)	P
Total	742	239	17	12.2764	0.75-0.90	3994	1276	114	52-7680	>0.995
Pooled x^2			1	0.1797	0.50-0.75			1	1.7012	0.10-0.25
Homo-geneity x^2			16	12. 0967	0.50-0.75			113	51.0668	>0.90

Table 2. Frequency of segregating and nonsegregating $S_{1,2}$ families of NJ89 -1

Segregating	Nonsegregating	x^2(2:1)	p
114	67	0.9454	0.25-0.50

Table 3. Allelism tests between NJ89-1 heterozygous plants with *Ms1ms1, Ms2ms2, Ms3ms3, Ms4ms4*, and *Ms5ms5*

		S_0 generation	
Cross	Year	No. sterile plants	No. fertile plants
NJ89-1 het. x *Ms1ms1*	1995	0	5
NJ89-1 het. x *Ms2ms2*	1995	0	6
Ms2ms2 x NJ89-1 het.	1995	0	5
NJ89-1 het. x *Ms3ms3*	1994	0	6
Ms3ms3 x NJ89-1 het.	1994	0	6
NJ89-1 het. x *Ms4ms4*	1995	0	4
Ms4ms4 x NJ89-1 het.	1995	0	3
Ms5ms5 x NJ89-1 het.	1995	0	3

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Primary Exploration on Formation of Differentiation of Growth Periods of Chinese Wild Soybean (*G. soja*) Affected by Geo-Ecological Factors

Abstract

When wild soybeans (*G. soja*) collected from different geo-ecological areas in Chinese mainland were planted in their local agricultural experiment stations from 1982-1983, their growth periods appeared to have great variation. This phenomenon was considered that they adapted their local geo-ecological environments: day length, temperature, rainfall, and so on selected by them and formed their growth periods and showed the diversity. The primary effect among the geo-ecological factors on the formation of wild soybean growth period was accumulative day length from emergence to bloom analyzed by stepwise regression and elimination. An equation was established to predict the possibilities of wild soybean growth periods in their natural adapted places.

Key words: wild soybean, geo-ecology, growth period

Annual wild soybean (*G. soja*) growing in moist or semi-moist areas of the template zone in East Asia is the ancestor of cultivated soybean (*G. max*). Geo-ecological factors: latitude, altitude, day length, rainfall, etc. within the region maintain great variations. Wild soybean species have been growing in such a complicated environment, permanently selected by them forming different genetically controlled growth types which would be the basis of responsiveness of cultivated soybean to light duration during growth seasons.

The purpose of this study is to evaluate effects of complicated geo-ecological factors on the formation of different types of growth periods, choose the most important one, and establish an equation to predict them.

Materials and Methods

120 accessions of wild soybean and semi-wild soybean (Table 1) collected from different latitude, longitude and altitude throughout the whole continent were planted in their nearest agricultural experiment stations from 1982-1983, repeated at least two times. Vegetative days (from emergence to bloom) and growth days (from emergence to maturity) were observed and recorded. Investigations of geo-ecological factors in different collected spots: latitude, longitude, altitude, accumulative day length 1, or D1, (from emergence to June 20th, before this day D1 value increased), accumulative day length 2, or D2, (from June 21st to maturity, after June 21st D2 value decreased) (Figure 1), annual effective accumulative temperature and annual rainfall, 7 geo-ecological factors were done. A data base was established by Foxbase, and curves of growth periods varied with geo-ecological factors were respectively pictured by HG computer software. Evaluation of functions of geo-ecological factors during the process of formation of growth periods of wild soybean was done, and an equation to predict its growth days in different collected spots was established by stepwise regression and elimination.

Results and Discussions

1. Effects of day length variation in different collected spots on their growth periods

While temperature was steady over 10°C in Spring, wild soybean emerged and daylight began affecting its growth and development. Before June 20th in a year, day length increased; but after June 20th day length decreased. Day length showed a sine curve variation within growth seasons (Figure 1). The lower the latitude, the gentler the sine curve.

As well known, wild soybean emerged and grew at the begining of April in low latitude areas, for example 30°N, at least until end of August it bloomed. Vegetative days were quite more and D1 value was high (Appendix I and Table 2 for materials).

On the contrary, wild soybeans growing in high latitude areas, for example 50°N, usually started growing at the middle or end of May and bloomed at the beginning or middle of July, vegetative days were much fewer and D1 value

was relatively low. Vegetative days increased as D1 value increased, as shown in Figure 2.

One problem should be pointed out that there was a differential, if bloom was after June 20th when D1 value was used to represent day length's effects on vegetative days of wild soybean. The near to June 20th bloom was, the less differential would be, so was for D2 value. In practice bloom moved to a later time with latitude decrease. If D1 from emergence to bloom and D2 from bloom to maturity were taken, D1 in low latitude will be higher and D2 value will be lower than now. Range of D1 from high to low latitudes (Table 2) will be much greater and that of D2 will be much more narrow.

The situation in reproductive stage was different. It usually was able to complete within 80 days in growth period of wild soybean at any place, from the lowest latitude to the highest one where wild soybean was growing. As mentioned above, the range of D2 value was more narrow (Table 2). As a result, its effect on growth period was less important.

Generally speaking, growth period of wild soybean increased with increase of D1 value (Figure 3).

2. Regulations of growth period varied with latitude

Regulative changes of blooms and maturities of experiment materials collected from different geo-ecological environments are shown in Table 3. Vegetative days and growth days increased as latitude decreased (Figures 4 and 5). Growth days appeared less discretion than vegetative days. The range of its coefficient of variation was fairly narrow than that of vegetative days comparing between their variations except materials within 27°00'-29°59' because of the effect of altitude which had a great change.

3. Effects of annual rainfall and annual effective temperature on growth period of wild soybean

As shown in Figure 6, growth days increased as annual rainfall increased. The same variation can be seen in Figure 7. That is growth days also increased as annual effective temperature increased. Annual effective temperature and rainfall in low latitude was much more than those in high latitude, as a result, growth days were delayed. However, variations of both of them were easy up. Changes of altitude also affected growth day. Growth days fairly increased when altitude was raised (Figure 8).

It is necessary to mention that growth period of wild soybean was a result of synthesized effects of geo-ecological factors, they coordinated each other, change of any one among them could result in growth days or other morphological changes.

4. Primary exploration of formation of growth period of wild soybean effected by geo-ecological factors

Evaluation of geo-ecological factors: D1, D2, annual effective temperature, annual rainfall, latitude, longitude, and altitude has been done through stepwise regression and elimination. An equation was established as following:

$$\hat{Y}=156.14150 + (-2.12176)X1 + (0.10143)X4 + (-0.01934)X7$$

$$R = 0.8763 \qquad Sr = 1.3440$$

XI: latitude X4: D1 value X7: annual rainfall
$\hat{Y}$: predict growth period

The most important ecological factor for the formation of growth days is D1, from emergence to bloom, which has the greatest affect on $\hat{Y}$ value according to the equation. Latitude has a secondary important role, then annual rainfall. D2, annual effective temperature, altitude and longitude were eliminated during the calculation process. This means that those factors played less important roles for growth days. Meanwhile, light intensity, light quality, wind and so on would also affect it in some degree also, and less important. They were not discussed here. Comparisons between observed and predicted data calculated by the equation are shown in Figure 9.

Another very important factor for flower is photoperiod. As we know, flowering would be advanced if shorter photoperiod was used with seedlings of wild soybean from low latitude because the photoperiod in low latitude was shorter than that in higher one as shown in Figure 1. This problem will be discussed in detail in further studies.

A remaining question for the research is how changes happened during the process from wild to cultivated soybeans and how different season types of cultivated soybeans being used in soybean production were formed, and how planting date was determined at one place. This is a critical problem for planting soybeans for normally growing and getting high yield. We plan further studies on *G. soja*.

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Table 1. Averages of major geo-ecology factors based on the collected spots located within ranges of latitude.

	matl.	Altitude		D1**		D2***		>10**** temp.		Rainfall	
Latitude	num.	Ave.	CV%*	Ave.	CV%*	Ave.	CV%*	Ave.	CV%*	Ave.	CV%*
48°00'-51°43'	8	233	25	470	3	1190	1	2350	7	441	15
42°00'-45°59'	7	124	27	714	10	1340	3	3119	5	591	14
40°00'-41°59'	13	402	72	782	9	1405	0	2891	12	614	24
38°00'-39°59'	15	653	119	857	2	1456	0	3434	15	499	34
36°00'-37°59'	23	537	103	891	4	1505	0	3787	15	585	26
34°00'-35°59'	33	481	95	949	4	1550	0	4122	17	647	15
32°00'-33°59'	10	381	96	983	6	1588	0	4540	7	876	21
30°00'-31°59'	8	202	158	1080	4	1625	0	5044	6	1153	12
27°00'-29°59'	3	925	126	1160	13	1649	0	4725	27	1124	35
sum.	120										

*: CV: Coefficient of Variation
**: D1: Accumulative day length hours from emergence to June 20th
***: D2: Accumulative day length hours from June 21st to maturity
****: >10 temp. Average of annual effective temperature

Table 2. Comparisons of growth periods of wild soybean growing in different adapted places

Origin		Lat.*	alt.* m.	D1* hur.	D2* hur.	vege.** days	reprd.** days	growth** days
Heilj***	Huma[1]	51.72°	178.2	441	1080	51	56	107
Heilj***	Nenj[2]	48.16°	243.0	477	1190	58	54	112
Heili***	Dedu	48.50°	273.2	472	1198	58	54	112
Heilj***	Harb[3]	45.75°	143.0	537	1225	55	65	120
Jilin	Shl[7]	43.50°	114.9	743	1267	66	64	130
Beijing		39 32°	40.0	862	1310	83	69	152
Shanx****	Dinx[4]	38.30°	960.4	883	1394	79	62	141
Shand*****	Jini[5]	35.13°	45.2	971	1498	103	76	179
Anhui	Wuhe	33.04°	21.0	960	1587	114	73	187
Hubei	Shnj[6]	31.26°	937.2	1081	1664	118	73	191

*: adapted from Meteorologic observation data on the earth's surface in China 1981-1990
Chinese meteorological bureau
**: adapted from Inventory of Chinese wild soybean, 1990 Institude of Crop Germplasm Resources
***: Heilongjiang province
1. Huma county
2. Nenjiang county
3. Harbin
****: Shanxi province
4. Dingxiang county
*****: Shandong province
5. Jining county
6: Shennongjia region
7: Shuangliao

Table 3. Vegetative and growth days of materials collected from the whole continent varied with latitude

Latitude	Materials num.	Vegetative days Average	Vegetative days CV*%	Growth days Average	Growth days CV*%
48°00'-51°43'	8	56	14	111	3
42°00'-45°59'	7	68	88	132	30
40°00'-41°59'	13	81	39	145	4
38°00'-39°59'	15	81	17	146	32
36°00'-37°59'	23	93	50	159	86
34°00'-35°59'	33	102	10	176	10
32°00'-33°59'	10	112	21	186	11
30°00'-31°59'	8	133	65	205	36
27°00-29°59'	3	130	53	194	133
sum.	120				

CV: Coefficient of Variation

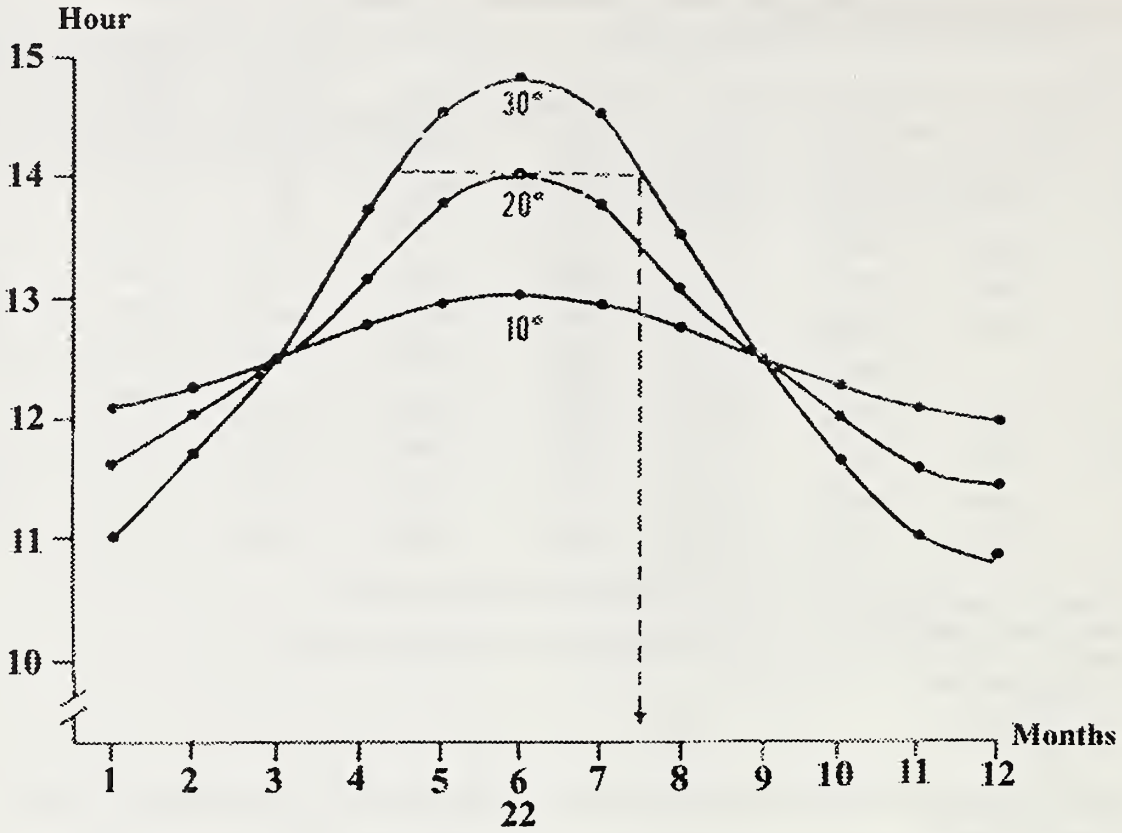


Figure 1. Variation of daylength with latitude in a year

FIGURE 2. VARIATION OF VEGETATIVE DAYS AS DAYLENGTH CHANGES

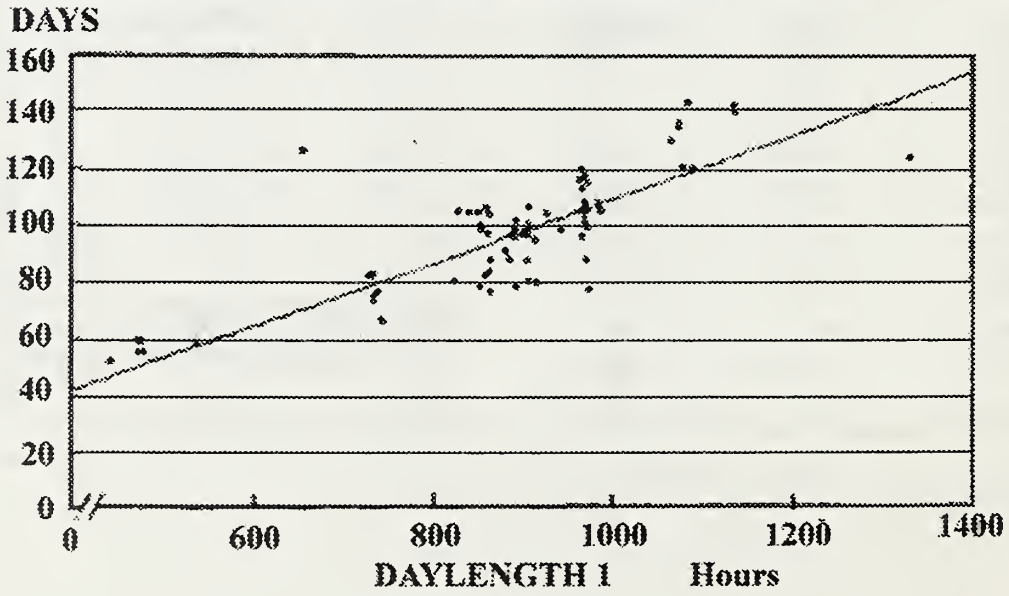


FIGURE 3. VARIATION OF GROWTH DAYS AS DAYLENGTH CHANGES

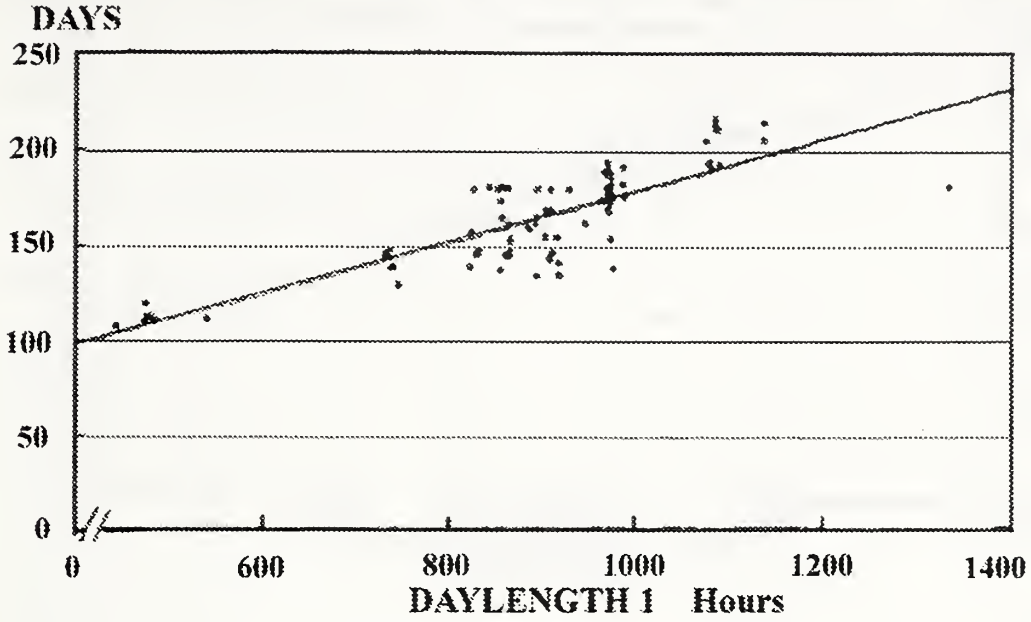


Figure 4. VARIATION OF VEGETATIVE DAYS AS LATITUDE CHANGES

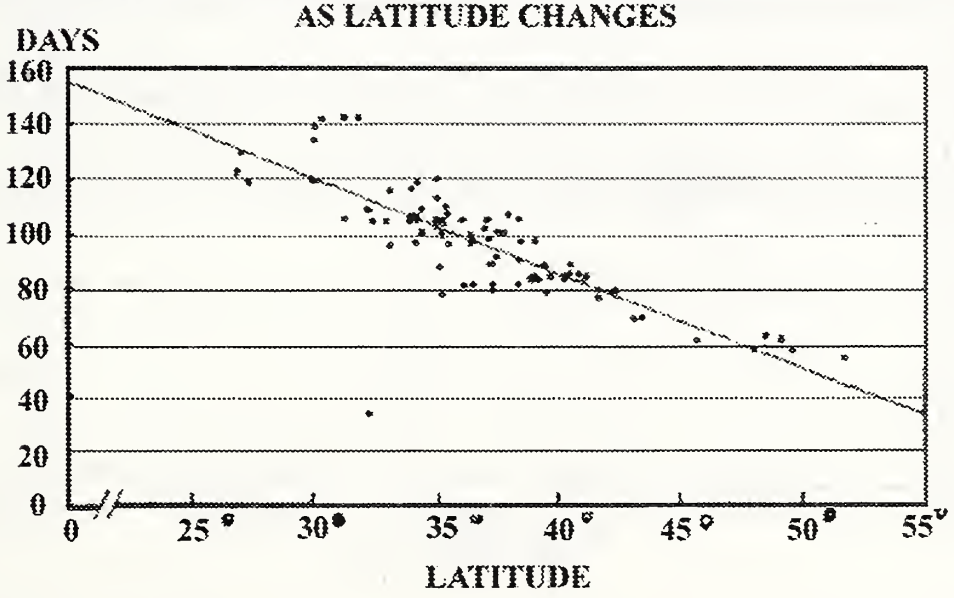


Figure 5. VARIATION OF GROWTH DAYS AS LATITUDE CHANGES

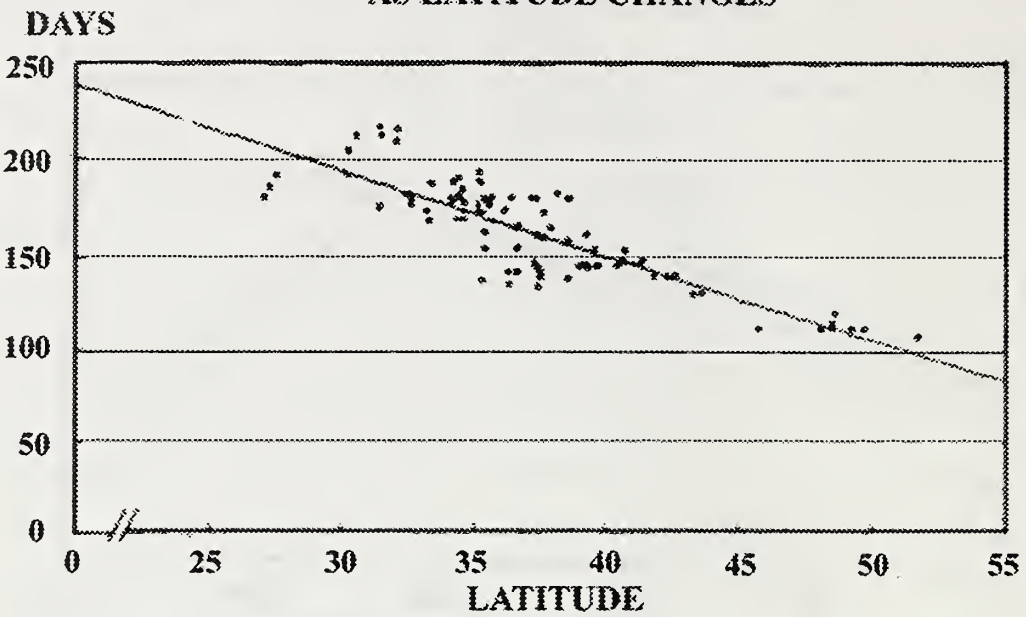


Figure 6. VARIATION OF GROWTH DAYS AS RAINFALL CHANGES

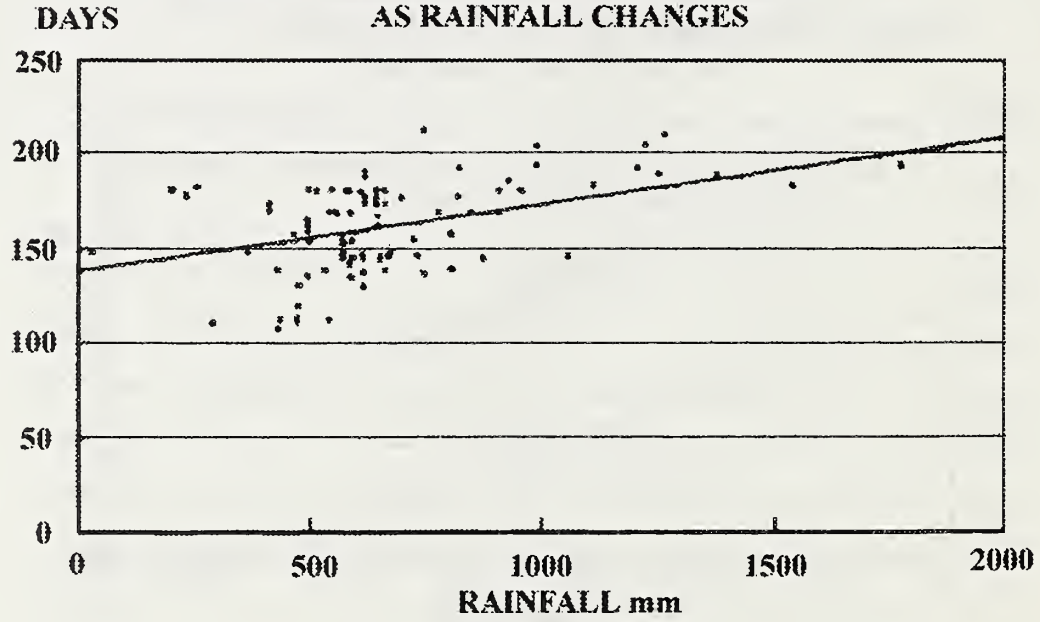


Figure 7. VARIATION OF GROWTH DAYS AS TEMPERATURE CHANGES

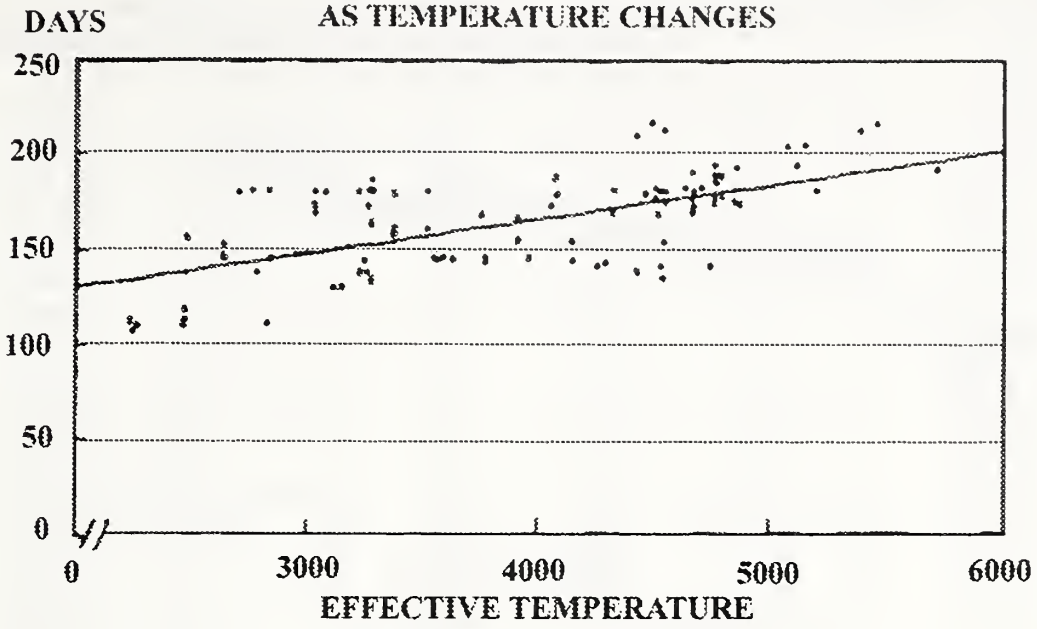


Figure 8. VARIATION OF GROWTH DAYS AS HEIGHT CHANGES

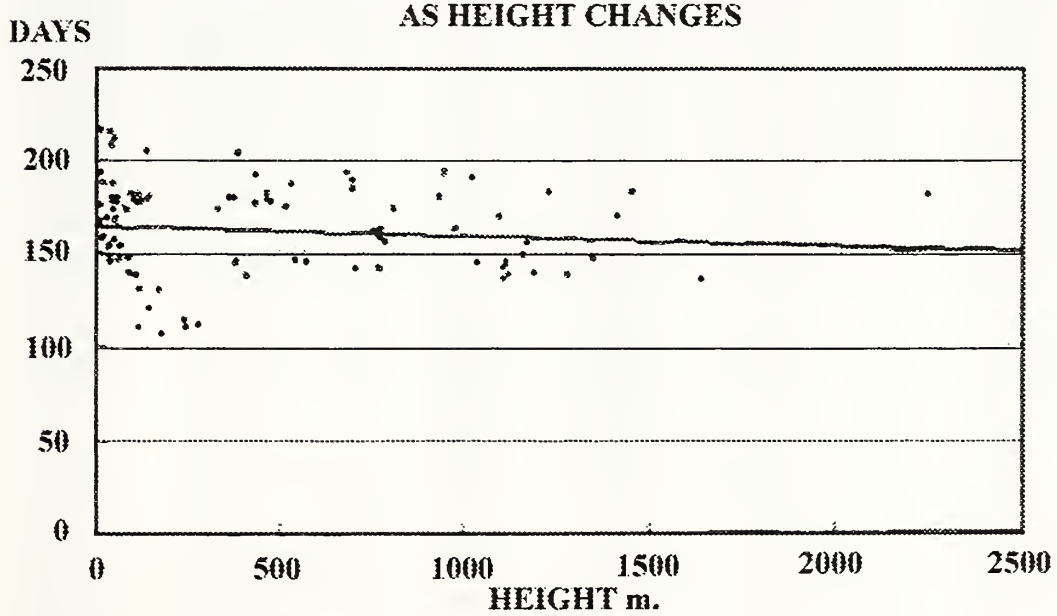
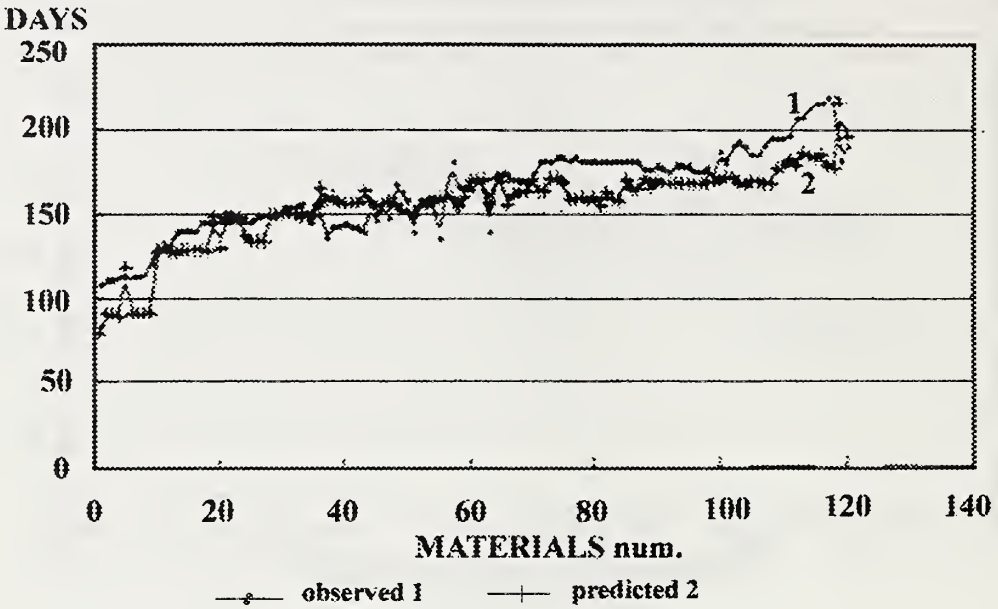


Figure 9. COMPARISON OF OBSERVED AND PREDICTED GROWTH DAYS



Appendix I

Vegetative and growth days of materials collected from different spots and replanted in their nearest agricultural experiment stations in 1982-1983, repeated two times,

Record #	Places	Lati-tude	Altit-ude	Longi-tude	Emergence	Bloom	Vege-tative	Maturity	Growth day
1	Huma	51.72	178.2	126.39	05/20/00	07/10/00	51	09/04/00	107
2	Xunke	49.58	113.0	128.28	05/18/00	07/11/00	54	09/05/00	110
3	Dedu	48.50	243.0	125.14	05/18/00	07/11/00	54	09/05/00	110
4	Dedu	48.50	273.2	126.11	05/16/00	07/13/00	58	09/05/00	112
5	Dedu	48.50	273.2	126.11	05/16/00	07/13/00	58	09/05/00	112
6	Dedu	48.50	273.2	126.37	05/16/00	07/13/00	58	09/05/00	112
7	Nenjiang	48.16	273.2	126.11	05/16/00	07/13/00	58	09/05/00	112
8	Keshan	48.05	236.3	125.53	05/16/00	07/14/00	59	09/06/00	113
9	Harbin	45.75	143.0	126.46	05/21/00	07/15/00	55	09/18/00	120
10	Shuanglao	43.50	114.9	123.32	05/13/00	07/18/00	66	09/20/00	130
11	Huaide	43.18	164.2	124.21	05/13/00	07/18/00	66	09/20/00	130
12	Huaide	43.18	164.2	124.21	05/13/00	07/18/00	66	09/20/00	130
13	Kaiyuan	42.53	99.0	124.03	05/05/00	07/18/00	74	09/20/00	138
14	Kaiyuan	42.53	99.0	124.03	05/05/00	07/20/00	76	09/20/00	138
15	Tieling	42.30	83.3	123.52	05/05/00	07/20/00	76	09/21/00	139
16	Tonghua	41.68	403.2	125.54	05/05/00	07/20/00	76	09/20/00	138
17	Longhua	41.32	562.3	117.43	05/02/00	07/22/00	81	09/23/00	144
18	Liaoyiang	41.20	30.5	123.04	05/02/00	07/22/00	81	09/23/00	144
19	Longhua	41.19	562.3	117.43	05/02/00	07/22/00	81	09/23/00	144
20	Luanping	40.93	535.8	117.20	05/02/00	07/23/00	82	09/24/00	145
21	Chengde	40.58	374.4	117.56	05/03/00	07/23/00	81	09/24/00	144
22	Chengde	40.58	374.4	117.56	05/02/00	07/23/00	82	09/24/00	145
23	Chengde	40.58	374.4	117.56	05/02/00	07/23/00	82	09/24/00	145
24	Chengde	40.58	374.4	117.56	05/02/00	07/23/00	82	09/24/00	145
25	Chengde	40.58	374.4	117.56	05/02/00	07/23/00	82	09/24/00	145
26	Gaixian	40.42	31.1	122.21	05/02/00	07/23/00	82	09/26/00	147
27	Neimenggu	40.38	1153.9	111.48	05/02/00	07/23/00	82	09/26/00	147
28	Xiuyan	40.28	80.8	123.17	05/02/00	07/24/00	83	09/26/00	147
29	Fuxian	39.70	60.0	122.00	05/02/00	07/24/00	83	09/26/00	147
30	Tongxian	39.55	26.9	116.38	05/02/00	07/24/00	83	10/01/00	152
31	Tongxian	39.55	26.9	116.38	05/02/00	07/24/00	83	10/01/00	152
32	Lugouqao	39.52	56.3	116.15	05/01/00	07/26/00	86	10/01/00	153
33	Changhai	39.27	30.5	122.35	04/30/00	07/25/00	86	10/01/00	154
34	Hequ	39.17	1032.0	111.16	05/05/00	07/20/00	76	09/26/00	144
35	Changhai	39.06	66.4	123.13	04/30/00	07/26/00	87	10/01/00	154.
36	Liushun	38.82	62.4	121.14	04/30/00	07/26/00	87	10/01/00	154
37	Pingluo	38.54	1099.9	106.33	05/06/00	07/24/00	79	09/18/00	135
38	Dingxiang	38.50	760.4	112.90	05/06/00	07/24/00	79	09/24/00	141
39	Dingxiang	38.50	760.4	112.90	05/06/00	07/24/00	79	09/24/00	141
40	Helan	38.46	1108.0	105.54	05/06/00	07/24/00	79	09/26/00	143
41	Wutai	38.43	1100.0	113.22	05/06/00	07/24/00	79	09/24/00	141
42	Wutai	38.43	700.0	113.22	05/06/00	07/24/00	79	09/24/00	141
43	Lingwu	38.07	1117.3	106.18	05/08/00	07/23/00	76	09/22/00	137
44	Linxian	37.90	1164.0	111.00	04/21/00	07/24/00	94	09/22/00	154

Record #	Places	Lati-tude	Altit-ude	Longi-tude	Emergence	Bloom	Vege-tative	Maturity	Growth day
45	Hengshan	37.56	1107.0	109.40	04/30/00	07/26/00	87	09/21/00	144
46	Taiyuan	37.47	779.5	112.33	04/21/00	07/25/00	95	09/23/00	155
47	Jingbian	37.37	1336.9	108.48	04/30/00	07/26/00	87	09/23/00	146
48	Kenli	37.36	9.6	118.32	04/21/00	07/27/00	97	10/04/00	166
49	Jiaocheng	37.32	760.2	112.07	04/21/00	07/26/00	96	09/29/00	161
50	Huimin	37.30	12.2	117.32	04/30/00	07/27/00	88	10/04/00	157
51	Zhongning	37.29	1184.9	115.40	05/05/00	07/23/00	79	09/20/00	138
52	Weihai	37.29	45.0	122.08	04/30/00	07/27/00	88	10/04/00	157
53	Wenshui	37.27	761.6	112.03	04/21/00	07/25/00	95	09/26/00	158
54	Mouping	37.23	17.0	121.35	04/28/00	07/27/00	90	10/04/00	159
55	Jingtai	37.11	1631.5	104.03	05/05/00	07/21/00	77	09/16/00	134
56	Jiexiu	37.02	745.8	111.54	04/21/00	07/26/00	96	09/28/00	160
57	Licheng	36.60	57.8	117.10	04/19/00	07/28/00	100	10/15/00	179
58	Laiyang	36.58	53.6	120.44	04/21/00	07/28/00	98	10/02/00	164
59	Laiyang	36.58	53.6	120.44	04/21/00	07/28/00	98	10/02/00	164
60	Wuxiang	36.50	965.5	112.51	04/21/00	07/27/00	97	09/30/00	162
61	Qihe	36.47	23.6	116.45	04/21/00	07/28/00	98	10/06/00	168
62	Pingdu	36.46	48.7	119.56	04/21/00	07/28/00	98	10/06/00	168
63	Huachi	36.27	1270.0	107.59	05/05/00	07/21/00	77	09/20/00	138
64	Pingyin	36.17	42.8	116.26	04/21/00	07/29/00	99	10/10/00	172
65	Pingyin	36.17	42.8	116.26	04/21/00	07/29/00	99	10/10/00	172
66	Anyang	36.07	76.4	114.22	04/21/00	07/29/00	99	10/10/00	172
67	Huanglong	35.60	1088.5	109.90	04/25/00	07/29/00	95	10/10/00	168
68	Huanglong	35.60	1088.5	109.90	04/25/00	07/29/00	95	10/10/00	168
69	Huanglong	35.60	1088.5	109.90	04/25/00	07/29/00	95	10/10/00	168
70	Huanglong	35.60	1088.5	109.90	04/25/00	07/29/00	95	10/10/00	168
71	Fanxian	35.42	38.0	115.25	04/18/00	08/01/00	105	10/14/00	179
72	Hejing	35.37	459.5	110.43	04/18/00	08/01/00	105	10/14/00	179
73	Feicheng	35.30	120.5	118.00	04/21/00	07/29/00	99	10/16/00	178
74	Zhenning	35.30	1443.4	108.21	04/18/00	08/01/00	105	10/16/00	181
75	Hancheng	35.28	458.8	110.27	04/16/00	08/01/00	107	10/14/00	181
76	Jining	35.26	45.2	116.35	04/20/00	08/01/00	103	10/16/00	179
77	Ningxian	35.25	1222.0	108.00	04/18/00	08/01/00	105	10/16/00	181
78	Wuzhi	35.06	96.1	113.24	04/20/00	08/01/00	103	10/16/00	179
79	Jiyuan	35.05	140.7	112.38	04/20/00	08/01/00	103	10/16/00	179
80	Jiyuan	35.05	140.7	112.38	04/20/00	08/01/00	103	10/16/00	179
81	Jiyuan	35.05	140.7	112.38	04/20/00	08/01/00	103	10/16/00	179
82	Jiyuan	35.05	140.7	112.38	04/20/00	08/01/00	103	10/16/00	179
83	Jiyuan	35.05	140.7	112.38	04/20/00	08/01/00	103	10/16/00	179
84	Jiyuan	35.05	140.7	112.38	04/20/00	08/01/00	103	10/16/00	179
85	Longxian	34.54	925.1	106.50	04/20/00	08/01/00	103	10/16/00	179
86	Yongji	34.53	355.0	110.27	04/20/00	08/01/00	103	10/16/00	179
87	Dali	34.52	368.9	109.56	04/20/00	08/01/00	103	10/16/00	179
88	Lankao	34.51	72.2	114.49	04/19/00	08/01/00	104	10/10/00	174
89	Mengjing	34.50	324.6	112.26	04/20/00	08/01/00	103	10/10/00	173
90	Zhenzhou	34.47	111.3	113.39	04/20/00	08/01/00	103	10/13/00	176
91	Ruicheng	34.42	507.1	110.43	04/20/00	08/01/00	103	10/11/00	174
92	Fengxian	34.41	40.6	116.35	04/23/00	08/02/00	101	10/12/00	172
93	Jingyang	34.33	428.3	108.49	04/20/00	08/01/00	103	10/13/00	176
94	Shangqiu	34.27	51.0	115.40	04/20/00	08/02/00	104	10/13/00	176

Record #	Places	Lati-tude	Altit-ude	Longi-tude	Emergence	Bloom	Vege-tative	Maturity	Growth day
95	XianYang	34.24	472.8	108.43	04/20/00	08/01/00	103	10/13/00	176
96	Luhua	34.12	800.0	111.20	04/20/00	08/02/00	104	10/10/00	173
97	Haoxian	34.09	327.5	112.05	04/20/00	08/02/00	104	10/10/00	173
98	Shuyang	34.06	8.8	118.45	04/20/00	08/05/00	107	10/12/00	175
99	Lixian	34.00	1405.4	115.11	04/25/00	08/02/00	99	10/10/00	168
100	Zhenan	33.26	693.6	109.09	04/18/00	08/03/00	107	10/19/00	184
101	Shenqui	33.24	42.0	115.04	04/19/00	08/02/00	105	10/12/00	177
102	Wuhe	33.08	21.0	117.53	04/10/00	08/02/00	114	10/14/00	187
103	Wenxian	32.57	1016.9	104.40	04/12/00	08/07/00	117	10/19/00	190
104	Fuyang	32.55	38.6	115.49	04/10/00	08/02/00	114	10/14/00	187
105	Queshan	32.48	85.5	114.02	04/18/00	08/03/00	107	10/17/00	182
106	Tanghe	32.41	109.9	112.51	04/18/00	08/03/00	107	10/17/00	182
107	Zhenba	32.30	694.3	127.52	04/16/00	08/05/00	111	10/21/00	188
108	Wanyuan	32.04	674.0	108.02	04/10/00	08/06/00	118	10/20/00	193
109	Fangxian	32.03	435.2	110.44	04/10/00	08/06/00	118	10/18/00	191
110	Shennong	31.45	937.2	110.40	04/10/00	08/06/00	118	10/18/00	191
111	Chongming	31.37	8.9	121.27	04/10/00	08/06/00	118	10/20/00	193
112	Pingchang	31.34	377.3	107.06	04/05/00	08/15/00	132	10/25/00	203
113	Yichang	30.42	134.3	111.18	04/04/00	08/20/00	138	10/25/00	204
114	Wuchang	30.21	38.2	114.19	04/04/00	08/22/00	140	10/30/00	209
115	Lingan	30.14	42.6	119.42	04/04/00	08/22/00	140	11/02/00	212
116	Hangzhou	30.14	43.2	120.12	04/04/00	08/22/00	140	11/02/00	212
117	Dinhai	30.02	36.6	122.06	04/04/00	08/23/00	141	11/06/00	216
118	Ruian	27.47	9.5	120.37	04/04/00	08/23/00	141	11/06/00	216
119	Ninglang	27.18	2242.3	100.51	04/19/00	08/18/00	121	10/15/00	179
120	Shibing	27.02	524.4	108.08	04/16/00	08/22/00	128	10/19/00	186

Appendix II

Vegetative and growth days of materials collected from different spots and replanted in Beijing in 1993, repeated two times.

Record#	Place	Latitude	Emergence	Bloom	Vegetative days	Maturity	Growthday
1	Huma	51.72	04/28/93	05/26/93	28	07/02/93	66
2	Xunke	49.58	04/28/93	05/25/93	28	07/09/93	73
3	Keshan	48.05	04/28/93	05/29/93	31	07/06/93	70
4	Neniiang	48.16	04/28/93	05/28/93	30	07/10/93	74
5	Harbin	45.75	04/30/93	06/06/93	37	08/14/93	107
6	Dedu	48.50	04/28/93	05/27/93	29	07/10/93	74
7	Dedu	48.50	05/07/93	06/06/93	30	08/03/93	88
8	Dedu	48.50	05/02/93	06/02/93	31	07/27/93	86
9	Dedu	48.50	05/02/93	06/01/93	30	07/10/93	69
10	Huaide	43.18	04/29/93	06/19/93	51	09/28/93	152
11	Shuanglao	43.50	04/30/93	06/24/93	55	09/28/93	151
12	Huaide	43.18	04/30/93	06/28/93	59	09/12/93	135
13	Tonghua	41.68	05/25/93	07/07/93	43	09/15/93	113
14	Kaiyuan	42.53	04/28/93	06/14/93	47	09/23/93	148
15	Kaiyuan	42.53	04/28/93	07/06/93	69	10/13/93	168
16	Tieling	42.30	04/28/93	06/17/93	50	09/16/93	141
17	Liaoyiang	41.20	04/28/93	06/13/93	46	09/16/93	141
18	Xiuyan	40.28	04/28/93	07/08/93	71	09/24/93	149
19	Liushun	38.82	04/28/93	07/08/93	71	09/02/93	127
20	Gaixian	40.42	05/06/93	07/17/93	72	10/04/93	151
21	Changhai	39.27	04/28/93	07/13/93	76	09/24/93	149
22	Changhai	39.06	04/29/93	08/03/93	96	10/09/93	163
23	Fuxian	39.70	05/19/93	08/14/93	87	09/28/93	132
24	Neimenggu	40.38	04/30/93	06/17/93	48	10/20/93	173
25	Luanping	40.93	04/28/93	07/06/93	69	09/10/93	135
26	Longhua	41.32	04/28/93	06/06/93	39	08/24/93	118
27	Longhua	41.19	04/29/93	06/09/93	41	09/10/93	134
28	Chengde	40.58	04/28/93	07/08/93	71	10/22/93	177
29	Chengde	40.58	04/28/93	07/01/93	64	09/12/93	137
30	Chengde	40.58	04/30/93	07/03/93	64	10/04/93	157
31	Chengde	40.58	04/28/93	06/21/93	54	09/29/93	154
32	Chengde	40.58	04/28/93	06/02/93	35	09/09/93	134
33	Tongxian	39.55	04/29/93	07/10/93	72	09/26/93	120
34	Tongxian	39.55	04/29/93	07/24/93	86	09/26/93	120
35	Lugouxiao	39.52	04/30/93	06/11/93	42	10/02/93	155
36	Jining	35.26	04/28/93	08/08/93	102	10/15/93	170
37	Pingyin	36.17	04/28/93	08/09/93	103	10/13/93	168
38	Pingyin	36.17	04/28/93	08/11/93	105	10/13/93	168
39	Qihe	36.47	04/29/93	08/24/93	117	10/20/93	174
40	Huimin	37.30	04/28/93	07/21/93	84	10/04/93	159
41	Licheng	36.60	04/28/93	07/29/93	92	09/28/93	153
42	Kenli	37.36	04/28/93	07/27/93	90	10/04/93	159
43	Feicheng	35.30	04/28/93	08/17/93	111	10/10/93	169
44	Pingdu	36.46	04/28/93	07/15/93	78	09/24/93	149
45	Weihai	37.29	04/28/93	07/15/93	78	10/07/93	162

Record#	Place	Latitude	Emergence	Bloom	Vegetative days	Maturity	Growthday
46	Laiyang	36.58	04/28/93	08/03/93	97	10/10/93	165
47	Mouping	37.23	04/28/93	08/03/93	97	10/02/93	157
48	Laiyang	36.58	04/28/93	07/29/93	92	09/29/93	154
49	Hequ	39.17	04/28/93	07/01/93	64	10/07/93	162
50	Wutai	38.43	04/28/93	07/04/93	67	09/24/93	149
51	Wutai	38.43	04/28/93	07/19/93	82	09/26/93	151
52	Dingxiang	38.50	04/28/93	07/08/93	71	09/24/93	149
53	Dingxiang	38.50	04/28/93	07/12/93	75	10/02/93	157
54	Taiyuan	37.47	04/28/93	07/21/93	84	10/07/93	162
55	Jiaocheng	37.32	04/28/93	07/05/93	68	10/05/93	160
56	Wenshui	37.27	04/28/93	07/10/93	73	09/28/93	153
57	Jiexiu	37.02	04/28/93	07/24/93	87	09/28/93	153
58	Linxian	37.90	04/28/93	07/06/93	69	09/17/93	142
59	Wuxiang	36.50	04/28/93	07/17/93	80	10/08/93	163
60	Hejing	35.37	04/30/93	07/29/93	91	10/19/93	173
61	Yongji	34.53	04/30/93	07/24/93	85	10/05/93	158
62	Ruicheng	34.42	04/29/93	07/29/93	91	10/05/93	159
63	Jingbian	37.37	04/30/93	07/08/93	69	09/12/93	135
64	Hancheng	35.28	04/29/93	08/24/93	117	10/22/93	176
65	Longxian	34.54	04/28/93	08/19/93	113	10/12/93	167
66	Hengshan	37.56	05/01/93	07/17/93	77	09/28/93	150
67	Zhenan	33.26	04/28/93	08/24/93	118	09/28/93	153
68	Huanglong	35.60	04/30/93	07/07/93	68	10/09/93	162
69	Huanglong	35.60	04/30/93	08/19/93	111	10/22/93	175
70	XianYang	34.24	05/01/93	08/24/93	115	10/17/93	169
71	Huanglong	35.60	04/30/93	08/17/93	109	10/22/93	175
72	Huanglong	35.60	04/30/93	07/24/93	85	10/14/93	167
73	Dali	34.52	04/30/93	08/26/93	118	10/22/93	175
74	Jingyang	34.33	04/28/93	07/27/93	90	10/12/93	167
75	Zhenba	32.30	05/01/93	08/24/93	115	10/20/93	172
76	Pingluo	38.54	04/28/93	07/01/93	64	09/21/93	146
77	Lingwu	38.07	04/28/93	07/17/93	80	10/04/93	159
78	Zhongning	37.29	04/30/93	07/10/93	71	09/24/93	147
79	Helan	38.46	04/28/93	06/06/93	39	10/12/93	167
80	Jingtai	37.11	04/29/93	07/10/93	72	09/29/93	153
81	Huachi	36.27	04/28/93	07/15/93	78	09/29/93	154
82	Lixian	34.00	04/28/93	07/10/93	73	10/08/93	163
83	Zhenning	35.30	04/28/93	07/20/93	83	09/29/93	154
84	Ningxian	35.25	04/28/93	07/24/93	87	10/07/93	162
85	Wenxian	32.57	04/28/93	08/24/93	118	10/22/93	177
86	Fengxian	34.41	04/28/93	07/24/93	87	09/28/93	153
87	Shuyang	34.06	04/29/93	08/08/93	101	10/06/93	160
88	Chongming	31.37	04/28/93	08/24/93	118	10/17/93	172
89	Wuhe	33.08	04/28/93	08/31/93	125	10/17/93	172
90	Fuyang	32.55	04/28/93	08/31/93	125	10/17/93	172
91	Anyang	36.07	04/29/93	08/19/93	112	10/13/93	167
92	Wuzhi	35.06	04/29/93	08/26/93	119	10/12/93	166
93	Fanxian	35.42	04/28/93	08/24/93	118	10/14/93	169
94	Ningyan	35.05	04/28/93	08/14/93	108	10/04/93	159

Record#	Place	Latitude	Emergence	Bloom	Vegetative days	Maturity	Growthday
95	Jiyuan	35.05	04/28/93	08/21/93	115	10/20/93	175
96	Jiyuan	35.05	04/28/93	08/19/93	113	10/21/93	176
97	Jiyuan	35.05	04/28/93	08/21/93	115	10/21/93	176
98	Lankao	34.51	04/28/93	08/21/93	115	10/04/93	159
99	Zhenzhou	34.47	04/28/93	08/24/93	118	10/17/93	172
100	Mengjing	34.50	04/28/93	08/24/93	118	10/22/93	177
101	Haoxian	34.09	04/28/93	09/02/93	127	10/22/93	177
102	Luhua	34.12	04/28/93	08/24/93	118	10/14/93	169
103	Shangqiu	34.27	04/28/93	08/17/93	111	10/22/93	177
104	Shenqiu	33.24	04/28/93	08/21/93	115	10/14/93	169
105	Queshan	32.48	04/28/93	08/27/93	121	10/22/93	177
106	Tanghe	32.41	04/30/93	08/24/93	116	10/19/93	172
107	Jiyuan	35.05	04/29/93	07/21/93	83	10/12/93	166
108	Jiyuan	35.05	05/03/93	08/03/93	92	10/04/93	154
109	Lingan	30.14	04/28/93	09/09/93	134	10/22/93	177
110	Ruian	27.47	04/29/93	09/16/93	140	10/22/93	176
111	Dinhai	30.02	04/29/93	09/11/93	135	10/19/93	173
112	Hangzhou	30.14	05/11/93	08/14/93	95	10/20/93	162
113	Wuchang	30.21	04/30/93	09/07/93	130	10/22/93	175
114	Fangxian	32.03	04/30/93	08/24/93	116	10/19/93	172
115	Shennong	31.45	04/28/93	08/31/93	125	10/22/93	177
116	Yichang	30.42	04/30/93	09/07/93	130	10/22/93	175
117	Wanyuan	32.04	04/28/93	08/31/93	125	10/22/93	177
118	Pingchang	31.34	04/29/93	09/11/93	135	10/22/93	176
119	Shibing	27.02	04/30/93	09/14/93	137	10/22/93	175
120	Ninglang	27.18	04/30/93	08/10/93	102	10/22/93	175

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RAPD and RFLP markers linked with the gene resistant to a SMV strain in China

Introduction

Soybean mosaic virus (SMV) is one of the most common diseases in soybean production in the world, resulting in serious yield reduction and seed-quality deterioration. A series of SMV strains have been recognized and the resistance sources have been identified in various areas and countries. Four major strains in the lower Changjiang valley were identified as Sa, Sc, Sg, and Sh. Resistance to the four strains were reported to be controlled by four linked single dominant genes *Rsa, Rsc, Rsg*, and *Rsh* (Gai et al., 1989; Xiang et al., 1991) Using RFLPs as genetic markers, virus-resistance genes have already been mapped in tomato and maize. RFLP and SSR were also used as genetic markers to identify chromosomal location of *Rsv*, a gene conferring resistance to SMV according to Yu et al. (1994). They constructed a F_2 population from a cross between soybean line PI 96983 as the resistant parent and cultivar Lee 68 as the susceptible parent. In the present study, we used RAPD and RFLP to identify the linkage between *Rsa* and molecular markers in order to provide some useful information for soybean breeding for disease resistance, and to make preparations for the mapping of molecular markers and the cloning of resistance genes.

Materials and Methods

1. Plant materials

A cross between Kefeng No. 1 and Nannong 1138-2 was made during 1994. Kefeng No. 1 is resistant to multiple strains such as Sa, Sc, Sg, Sh (they are prevailing in southern China); N1, N2, N3 (in Northeast China). While Nannong 1138-2 is susceptible to all these strains. F_1 plants were grown in a net-house in Hainan Island during the following winter, and were selfed to produce F_2 seeds. Parent plants (30), F_1 plants (30), F_2 plants (213), and $F_{2:3}$ lines (67 from resistant F_2 plants) were evaluated for response to SMV strain Sa. Once the first trifoliate leaves were partially expanded, unifoliate leaves were inoculated with Sa maintained on Nannong 1138-2. One to two weeks after inoculation, mosaic symptoms developed fully in newly formed leaves, and reactions to Sa of the individual plants were recorded as resistant (symptomless) or susceptible (mosaic).

2. DNA extraction pool making and primer screening

Total genomic DNAs were extracted using the CTAB method. Fifteen resistant F_2 individuals and 15 susceptible F_2 individuals were selected. Equal volumes (μg) of DNA from each individual were pooled to obtain two bulks. Four hundred and twenty decamer primers (Operon Technologies, Inc. Alameda, CA) were used (Table 1) to screen the two bulks. RAPD markers were amplified by using single oligonucleotide primers and DNAs from the bulks and their parents as templates. RAPD analysis was conducted according to Operen 10-mer kits' product information.

3. RFLP analysis

A sample of approximately 5 μg of DNA was individually digested with one of 5 restriction enzymes (Taq I, Dra I, Hind III, Eco RV and Bam H1). Restriction fragments were separated on 0.8% agarose gels according to standard electrophoresis procedures. DNA was transferred to nylon membrane via Southern blotting. Blots were hybridized with randomly primed ^{32}P-labeled dCTP insert DNA (pK644 was provided by Dr. Shoemaker and maintained in the Laboratory of Plant Biotechnology attached to the Institute of Genetics, Academia Sinica). Then exposed to x-ray film for 5-10 days.

Results and Discussion

The results from the inoculation experiment (Table 2) showed that the segregation of resistant to susceptible in F_2 fitted well the 3:1 ratio, the segregation ratio of resistant F_2, derived lines in F_3 fitted well, 1 completely resistant to 2 segregating. The segregation of resistant to susceptible of the population of segregating F_3 lines also fitted well the

3:1 ratio, which demonstrated the resistance to Sa were controlled by a single dominant gene *Rsa*.

The diversity in molecular level between Kefeng No. 1 and Nannong 1138-2 was quite wide suggested by RAPD and RFLP. Twenty primers (OPH-01--OPH-20) produced 145 bands, of which 25 bands (17.2%) were polymorphic between two parents. The polymorphic bands were produced with nine primers, they accounted for 45% of the primers used. When DNA were digested with Taq 1, Dra 1, Hind III, EcoR V and BamH 1, pK644, as a probe, polymorphisms could be seen between parents in any case.

When polymorphic bands contrasting for *Rsa* locus bulks were compared with the patterns generated in parents Kefeng No.1 and Nannong 1138-2, five polymorphic bands fitted the Kenfeng No.1-resistant bulk pattern VS. 1138-2-susceptible bulk. The five bands were generated by OPAF-20, OPAO-20, OPAS-06, OPAV-07, OPAW-07. Among them, OPAS-06_{1800} appeared to be best repeatable. The polymorphic DNA fragment OPAS-06_{1800} generated by OPAS-06 co-segregated with *Rsa* at a recombination value 23±6.29% (Table 3).

OPAS-06_{1800} amplified from Kefeng No.1 was retrieved with Micro-pipettes and was reamplified using primer OPAS-06 under the similar conditions described above. The re-amplified DNA sample was labeled with ^{32}P and used as a hybridization probe. Southern blotting results showed that it hybridized to single or low copy DNA.

A polymorphic RFLP marker, pK644H (pK644H/Hind III) was also identified when pK644 was used as a probe with the restriction enzyme Hind III. The marker co-segregated with *Rsa* in the F_2 population (50 plants), and the recombination value between them is 31±8.15% (Table 3). pK644H co-segregated with OPAS-06_{1800}, with a recombination value of 37±9.09%. The order of OPAS-06_{1800} *Rsa* and pK644H is indicated in Figure 1.

A cluster of resistance genes does exist according to earlier studies. Gai et al. (1989) and Xiang et al. (1991) reported that the resistance to SMV strains Sa, Sc, Sg, and Sh were conditioned by *Rsa, Rsc, Rsg,* and *Rsh*, respectively, the four dominant genes were on the same linkage group (Figure 2). Therefore, OPAS-06_{1800} and pK644H might also be on the same linkage group. Therefore, whether OPAS-06_{1800} is tightly linked with *Rsh* or *Rsc*, and whether pK644H is near to Rsh or Rsc rather than to *Rsa* are left to be studied in the future.

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Table 1. 420 decamer primers were used.

OPH 01-20	OPAA 01-20	OPAB 01-20	OPAC 01-20
OPAD 01-20	OPAE 01-20	OPAF 01-20	OPAH 01-20
OPAI 01-20	OPAJ 01-20	OPAK 01-20	OPAL 01-20
OPAM 01-20	OPAN 01-20	OPAO 01-20	OPAP 01-20
OPAS 01-20	OPAT 01-20	OPAV 01-20	OPAW 01-20
OPAX 01-20			

Table 2. Verification of single dominant gene resistant to Sa.

	No. of plants (lines)		Expected		
Generation	R	S	ratio	χ^2	p
♀Kefeng No. 1	30	0			
♂Nannong 1138-2	0	30			
F_1	30	0			
F_2	156	57	3:1	0.264	0.50-0.75
$F_{2:3\#}$	23	44(seg.)	1:2	0.002	>0.90
Fs*	424	163	3:1	2.253	0.10-0.25

derived from resistant F_2 plants
* plants from segregating F_3 lines

Table 3. Linkage relationship among *Rsa,* OPAS-06_{1800} and pK644H

Locus and	No. of plants							Recombination
markers	1,1	1,0	0,1	0,0	total	χ^2	p	value
Rsa-OPAS-06_{1800}	38	8	5	10	61	15.09	<0.005	23±6.3
Rsa-pK644H	29	8	6	7	50	6.25	0.10	31±8.2
OPAS-06_{1800}-pK644H	27	8	8	6	49	3.14	0.25-0.5	37±9.1

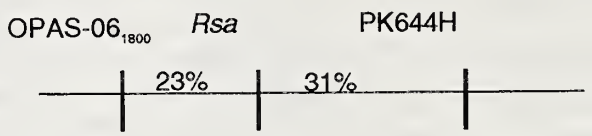

Figure 1. The order of *Rsa* and a RAPD and a RFLP markers

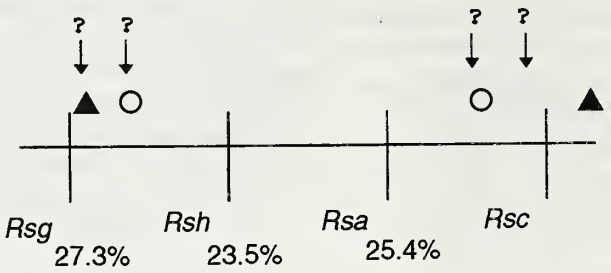

Figure 2. The possible position of OPAS-06$_{1800}$ (○) and pK644H(▲)

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Salt Tolerance of Wild Soybean (*Glycine soja*) in Natural Populations Evaluated by a New Method

Introduction

Improvement of crops more and more depends on exploitation of genetic resources of wild relatives. Soybean was generally considered to be a salt sensitive plant, however its wild relative *Glycine soja* has been found around the sea shore of North China. Salt tolerance in natural populations was previously evaluated by a salt tolerant index (Hannon, Bradshaw 1968). Preliminary tests showed that the method routinely used in perennial grasses can not be used for evaluation of salt tolerance of *Glycine soja* because of diversity of growth rate within populations and among populations. There were some reports on salt tolerance of soybean cultivars and perennial *Glycine* (Pantalone III and Kenworthy, 1989; Ragab et al., 1994) based on a comparison of growth rates in solution cultures with different NaCl concentrations. This method may not be proper to use when a single plant evaluation is needed in the case of bulk segregation analysis (Michelmore et al., 1991). According to Munns (1993) the timing and extent of leaf injury as a screening technique is also valid. Now we report some results on salt tolerance of *Glycine soja* in saline and normal populations by using a new method based on the timing of leaf injury.

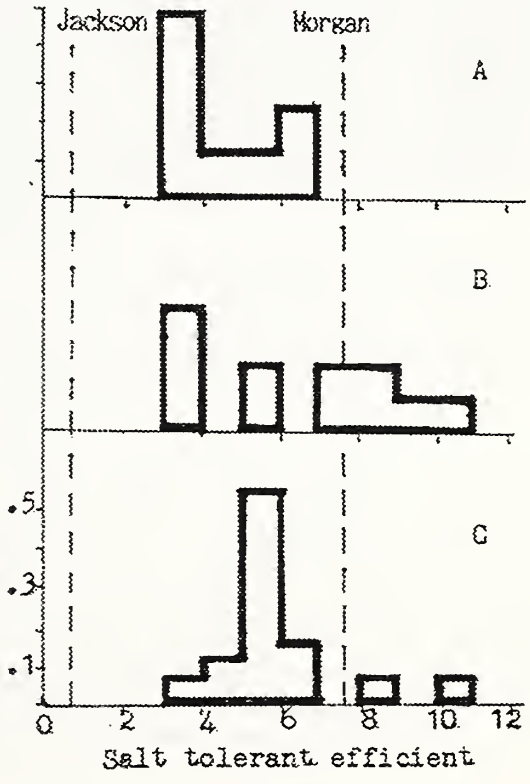
Materials and Methods

Wild soybean seeds of two saline populations were collected from Kenli county of Shantong Province where the last delta of the Yellow River has been formed from sea since 1976. A normal population was selected at the Yellow River bank near the urban area of the county. After germination, seedlings were first grown in Johnson's solution for one week then transferred into the same solution with added 0.2% NaCl and stayed for 5 days. After that NaCl concentration increased to 0.4% and the seedlings were grown for another 5 days. In the same way, the final concentration of NaCl in solution culture reached 0.8%. As soon as the plants showed typical leaf injury from salt, they were removed from salt solutions and transferred into normal solution without NaCl and recorded the days before the injury appeared. Salt tolerant efficiency was measured as a sum of days multiplied by concentration of NaCl added. For instance, leaf injury of cultivar Jackson appeared in the fourth day after growing in solution with supplement of 0.2% NaCl, therefore, the efficiency for Jackson was estimated as 0.2 x 3 = 0.6 which indicated salt sensitive. Leaf injury of cultivar Morgan occurred on the third day after growing in 0.8% NaCl, the salt tolerant efficient of Morgan was calculated as 0.2 x 5 + 0.4 x 5 + 0.6 x 5 + 0.8 x 2 = 7.6 which agreed with the highest level of salt tolerance (Ragab et al., 1994). In most cases, leaf injury for a given cultivar or accession occurred in same days. Experimental variability of a few accessions was around 1 to 2 days.

Results and Discussion

Salt tolerance of wild-soybean in three populations was shown in Figure 1.

Figure 1.



A. Normal Population
B. C. Saline Populations

The levels of salt tolerance for individuals within populations varied widely which agreed with other reports on salt tolerance of perennial plants (Kik, 1989; Wu, 1981). It indicated a higher level of salt tolerance in saline populations of wild soybean. In the saline populations there were some plants which tolerant level was much higher than that of all plants in the normal population, even higher than the tolerant level of the most salinity tolerant soybean cultivar Morgan. This result showed salt tolerance was a kind of adaptive characters, germplasms of high salt tolerance can be found in natural saline populations.

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Search for Molecular Markers of Salt Tolerance of Soybean by DNA Amplification Fingerprinting

Introduction

Genetic improvement of cultivated soybean requires a large amount of information about polymorphic markers linked to useful characters. However, strict inbreeding of soybean leads to low genetic diversity as most cultivars share a common ancestry and individuals are highly homogeneous. This low molecular diversity has been demonstrated by the difficulty to detect DNA polymorphisms (Doyle et al., 1988; Keim et al., 1989). Recently a new strategy based on arbitrary primer amplification (DAF) was used for identity testing, population and pedigree analysis (Prabhu et al., 1997), molecular characterization of near-isogenic lines, and high density genetic mapping (Prabhu and Gresshoff, 1994; Caetano-Anollés et al., 1993). In this study, we applied DAF to screen two salt-sensitive soybean cultivars (Hark and Jackson) and two salt-tolerant soybean cultivars (Morgan and Wenfeng No. 7) with the hope to find interesting polymorphic markers.

Materials and Methods

Total DNA was isolated from the leaves of soybean seedlings as described by Dellaporta et al. (1983). DNA amplification was performed in a solution with a total volume of 20 µL containing 2 ng of template DNA, 3 µM of primer, 6.0 U of AmpliTaq DNA polymerase (Stoffel fragment) (Perkin-Elmer, Norwalk, Conn.), 2.0 µL (each) of the four deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, and 10 mM Tris-HCl (pH 8.0). Samples were amplified in an Ericomp twinblock thermocycler (Ericomp Inc., San Diego, Calif.) connected to a refrigerated water bath for 35 three-step cycles of 30 s at 96°C, 30 s at 30°C and 30 s at 72°C, and 1 cycle at 72°C for 5 minutes. Amplified products (3 µL) were separated on 4.5% polyacrylamide gels and detected by silver-staining (Bassam et al., 1991). Data presented in Table 1 were scored by visual observation.

Results and Discussion

Eight oligonucleotide long primers (8-mers) were used to screen DNA from soybean cultivars Morgan, Jackson, Hark, and Wenfeng; thirteen polymorphic markers were obtained (1.6 amplification polymorphism (APs) per primer); seventy-five percent of used primers gave polymorphic bands. Compared with the data of RAPD polymorphisms obtained by Williams et al. (1990) using similar *Glycine* species as used in this study (0.5 APs/primer), DAF showed an increase in sensitivity probably caused by detection methods; the agarose/ethidium bromide method produces about 5 to 15 bands while acrylamide/silver-staining can detect 20-40 bands (Table 1).

Crosses were made between Wenfeng (♂) and Hark (♀), Jackson (♂) and Morgan (♀). One AP (350 bp) which was amplified using primers 8.6f was present in Wenfeng, absent in Hark, and failed to be detected in the F_1; another AP (170 bp), amplified using primer 8.7g was present in Jackson, absent in Morgan, and failed to be detected in F_1s. Because most DAF APs are dominant (Prabhu and Gresshoff, 1994), those putative F_1 plants were not genuine. This idea was confirmed by morphological characters. Hark developed purple flowers while Wenfeng showed white flowers, but no segregation occurred in all putative F_2 progenies. Accordingly, we conclude that all putative F_1 plants were products of self-fertilization.

The most interesting thing was that three polymorphic markers (8.6f/350 bp, 8-27/240 bp, 8-15/215 bp) only appeared in cultivars Morgan and Wenfeng. Further study is required to check where they are linked to the salt-tolerance character of soybean.

These results show the utility of dominant molecular markers in fast analysis of the F_1 status of putative crosses. Significant time was saved because progeny material did not need to be screened (for example, in physiological trials or bulked segregant analysis). DAF, because of its broad applicability to many crop species and the ease of gel detection, becomes another tool in the hand of the plant breeder and geneticist.

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Table 1. DNA amplification fingerprinting of soybean cultivars:

Primer code	Primer sequence 5' → 3'	No. of amplification products detected	% amplification polymorphisms (APs)
8.6 a	GAGCCTGT	29	0.0
8.6 d	GTAACGCC	40	2.5
8.6 e	GACGTAGG	35	5.7
8.6 f	GTACGCAG	29	10.3
8.6 l	GTAACCCC	20	15.0
8.7 e	CCTGGTGG	31	0.0
8.7 g	CCAGGTGG	27	7.4
8.7 h	CCTCGTGG	35	8.6

1) Primer code defines the length of the primer, its GC content and its order in the series. For example, primer 8.6 is an 8-mer with 60 to 69% GC, being first in the series.
2) As scored by visual comparison of bands between 100 and 700 bp on silver-staining gels.
3) Equivalent to number of APs per total number of resolved bands.

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Selection Effects of Soil Fertility on the Progenies of Soybean Crosses

Abstract

The F_2, F_3, and F_4 lines of six soybean crosses were selected successively under high- and low-fertility sites with the objective of high yield and the method of pedigree. Two best F_4-derived lines were chosen from each of the six crosses under both high- and low-fertility for use in this study. In 1995, the total 24 lines were tested in high-, medium-, and low-fertility sites with the same experimental design (CRB) to study the selection effects of high- and low-fertility. The results suggested that high- and low-fertility had different selection effects. High fertility was more effective for selecting lines which had higher yield under high-fertility and lower yield under low-fertility; low-fertility was better for selecting lines which had higher yield under low-fertility and lower yield under low-fertility; high-fertility was somewhat better than low-fertility for selecting lines which had higher yield under both high- and low-fertility. It revealed that the lines selected from high-fertility had superior yield potential. The lines selected from high-fertility had shorter plant height, more nodes on main stem, fewer branches, and higher seed-stem ratio. The reverse was true for the lines selected from low-fertility. The lodging-resistance of the lines selected from high-fertility was greater than that of the lines from low-fertility. The soil fertility level of breeding nursery should be chosen according to the breeding objective.

Key Words: progenies of soybean crosses, soil fertility, selection effects

Introduction

Plant breeders have been interested in the conditions of soil fertility because there are different selection effects among the different soil fertility. Different types of varieties can be bred from the same cross progeny if the progeny was selected under different soil fertility (Wang et al., 1962; Meng et al., 1986; Gotoh et al., 1959). The selection effects provide important basis for the choosing of fertility conditions of breeding nursery. The breeding efficiency will be high if the available soil fertility is used in nursery. Several studies (Wang et al., 1962; Meng et al., 1986; Yang et al., 1992; Frey, 1964; Gotoh et al., 1959; Whitehead et al., 1989) have considered the selection effects of soil fertility, however, soil fertility and other ecological conditions were usually not studied separately, and the results also had much difference. In order to reduce the influence of other ecological factors, the experiment was conducted in three locations which were all very near. The major objective of this study was to identify and estimate the selection effects of soil fertility to provide basis for the fertility choosing of breeding nursery.

Materials and Methods

The F_2, F_3, and F_4 lines of six soybean crosses, made by the Institute of Soybean of Northeast Agricultural University, were selected successively under high- and low-fertility sites with the objective of high yield and the method of pedigree. Two best F_4-derived lines were chosen from each of the six crosses under both high- and low-fertility for use in this study. In 1995, the total 24 lines were planted in the field of the Chicken Farm of Xiangfang Experiment Station, the breeding nursery of Xingfang Experiment Station, and Northeast Agricultural University, representing high, medium, and low fertility levels, respectively (Table 1). The three fields were all in the Harbin district. The experimental design was a randomized complete block with three replications. Plot size consisted of two rows that were 4.00-m long with 0.70-m spacing between rows and 0.10-m apart in the row. Main agronomic characteristics were recorded on the ten plants which were harvested from each plot at random. In addition, yield of each plot (5.20 m^2) was recorded.

Results and Discussion

Selection effects of soil fertility on the number of different kinds of lines

The mean yield of each line under high-fertility site was plotted against its mean yield under low-fertility site. The graph was then divided into four quadrants. The dividing lines being the grand mean of all the soybean lines (Y = 2196.97 kg ha^{-1}, horizontal line) and the grand mean of the soybean lines under high-fertility (X=3342.10 Kg ha^{-1}, vertical line, Figure 1). The lines with mean yields above the grand mean for both high- and low-fertility were called Both-Higher-Lines (BHL) (upper right-hand quadrant, Figure 1) in this study. The lines with mean yield above the grand mean for low-fertility but below the grand mean for high-

fertility were called Low-Fertility-Adaptive-Lines (LFAL) (upper left-hand quadrant, Figure 1). The lines with mean yield below the grand mean for both high- and low-fertility were called Both-Lower-Lines (BLL) (lower left-hand quadrant, Figure 1). The lines with mean yield above the grand mean for high-fertility but below the grand mean for low-fertility were called High-Fertility-Adaptive-Lines (HFAL) (upper right-hand quadrant, Figure 1).

As shown in Figure 1 and Figure 2, among the seven BHL, four lines (57.9% of the BHL) were selected from high-fertility site and three lines (42.9%) were selected from low-fertility site; Among the five LFAL, only one line (20.0%) was selected from high-fertility, all the other lines (80.0%) were selected from low-fertility; Among the six HFAL, four lines (66.7%) were selected from high-fertility, and two lines from high-fertility; Among the six BLL, half were from high-fertility and the other half were from low-fertility.

The above results revealed that low-fertility was more effective for selecting LFAL, high-fertility was more effective for selecting HFAL, and high-fertility was somewhat better than low-fertility for selecting BHL.

Selection effects of soil fertility on the yield of the progenies of soybean crosses

Variance analysis suggested that there were significant differences in soybean seed yield among the lines at 0.01 level ($F=34.41$, $F_{0.01}=1.94$). Moreover, the interaction of line x location was significant at 0.01 level ($F=34.16$, $F_{0.01}=1.62$). The results indicated that there were significant differences, which were significantly influenced by the soil fertility level, in seed yield among different lines. In high-fertility location, the grand mean yield of the lines (3453.84kg ha^{-1}) selected from high-fertility site was significantly higher than that (3230.21kg ha^{-1}) selected from low-fertility ($t=3.52$, $t_{0.05}=2.07$). In the low-fertility location, the grand mean yield of the lines (2164.06kg ha^{-1}) selected from high-fertility site was lower than that (2229.78kg ha^{-1}) selected from low-fertility, however, the difference wasn't significant at 0.05 level ($t=1.61$, $t_{0.05}=2.07$). It revealed that the lines selected from high-fertility had greater yield potential.

Selection effects of soil fertility on the plant type of the progenies of soybean crosses

Under high-, medium-, and low-fertility, the values of selection difference were all positive (Table 2). It suggested that the lines selected from low-fertility had higher plant height than those from high-fertility. The values of selection difference of the number of branches were 0.32, -0.01 and 0.30, respectively. The general trend was that the lines selected from low-fertility had more branches. It was reverse for the seed-stem ratio. Under the three kinds of fertility, the values of selection differences were -0.09,-0.02 and -0.10, respectively. It suggested that the lines selected from low-fertility had lower seed-stem ratio. The selecting trend of nodes on the main stem was the same as that of seed-stem ratio. The lines selected from low-fertility had fewer nodes on the main stem, for the values of selection difference were -0.73, -0.20 and -0.64, respectively.

The above results revealed that the plant types of the lines selected from high-fertilitv was less luxuriance with shorter plant height, more nodes on main stem, fewer branches and higher seed-stem ratio. The reverse was true for the lines selected from low-fertility.

Selection effects of soil fertility on the lodging-resistance of the progenies of soybean crosses

The lodging grades were different for the lines selected from high- vs. low-fertility (Figure 3). The lines selected from high-fertility were more resistant to lodging. While planted in the low-fertility site, all the lines were not lodging or just lodging slightly. It was difficult to identify the lodging-resistance difference among the lines in such condition. On the contrary, while planted in the high-fertility, all the lines were lodging to a greater or lesser extent. The significant difference provided the basis for the selection of the lines with lodging-resistance. Therefore, it is necessary to have a high-fertility nursery to identify and evaluate the lodging-resistance of the soybean lines.

Conclusion

Soil fertility in breeding nursery played an important role in the selection procedure of the progenies of soybean crosses. Results of the study showed that there were important selection effects of soil fertility on the number of different lines, the plant types, the seed yield and the lodging-resistance of the progenies of the soybean crosses. It suggested that the high-fertility nursery was more effective for selecting HFAL and the low-fertility nursery was better for selecting LFAL, furthermore, the high-fertility nursery was somewhat better for selecting BHL and lines with greater yield potential, identifying and evaluating the lodging-resistance of the soybean lines. On the basis of these results, it is recommended that the soil fertility level in breeding nursery should be chosen

according to the breeding objective. If the objective was to breed for low-fertility environment, it was better to choose the low-fertility as the nursery. If the objective was to breed for high-fertility environment, then it was better to choose high-fertility site as the nursery. It would be better to choose the high-fertility site as the nursery if the objective was to breed the varieties, which had higher yield in both high- and low-fertility environment.

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Table 1. Main soil fertility index of the three locations.

Location	Complete nitrogen (%)	P_2O_5 (%)	Slowly available potassium (ppm)	Alkali hydrolysis nitrogen (mg/100g)	Quickly available phosphate (ppm)	Quickly available potassium (ppm)	Organic matter (%)	pH value
HF	0.191	0.160	983.82	14.95	72.90	145.53	4.27	7.25
MF	0.144	0.147	872.58	12.08	66.07	133.17	2.23	7.20
LF	0.137	0.103	869.49	12.95	33.32	136.26	2.19	7.12

HF = high fertility, MF = medium fertility, LF = low fertility

Table 2. Selection effects of soil fertility on main agronomic characters.

Location for test	Location for selection	Lodging grade	Dry weight on ground (g)	Plant height (cm)	Nodes on main stem	Branches	Pods on main stem	Seeds per plant	100-seed weight (g)	Seed-stem ratio
HF	Mean of HF	3.61	65.67	91.06	17.51	3.71	51.85	142.69	18.23	0.80
	Mean of LF	4.14	63.93	92.93	16.78	4.03	49.91	131.89	18.45	0.71
	Value of selection difference	0.53	-1.74	0.87	-0.73	0.32	-0.94	-10.8	0.22	-0.09
MF	Mean of HF	1.40	59.96	84.00	17.10	3.48	46.33	147.09	16.22	0.72
	Mean of LF	1.60	52.84	87.30	16.90	3.68	50.82	142.37	16.09	0.70
	Value of selection difference	0.20	-4.12	3.30	-0.20	0.20	4.49	-4.72	-0.13	-0.02
LF	Mean of HF	1.04	39.50	68.63	16.60	2.00	40.60	94.40	17.10	0.70
	Mean of LF	1.08	41.10	70.05	15.96	2.30	42.00	102.0	16.60	0.60
	Value of selection difference	0.04	0.60	1.41	-0.64	0.30	1.40	7.6	-0.5	-0.10

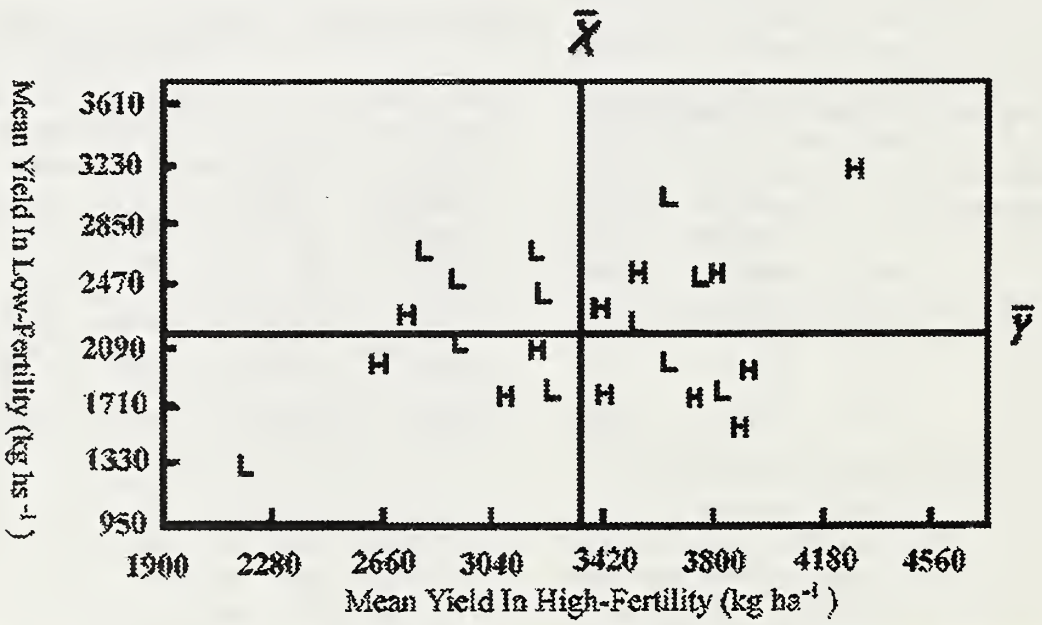

Figure 1. Scatter diagram of soybean lines selected from high-vs. low-fertility nursery

H = Lines selected from high fertility L = Lines selected from low fertility

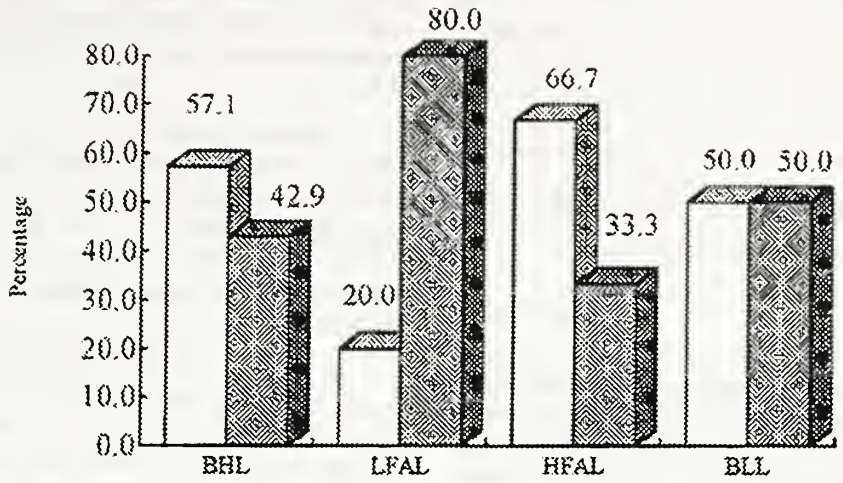

Figure 2. Selecting effects of high vs. low fertility on number of different kinds of lines

□ = Lines selected from high fertility

▨ = Lines selected from low fertility

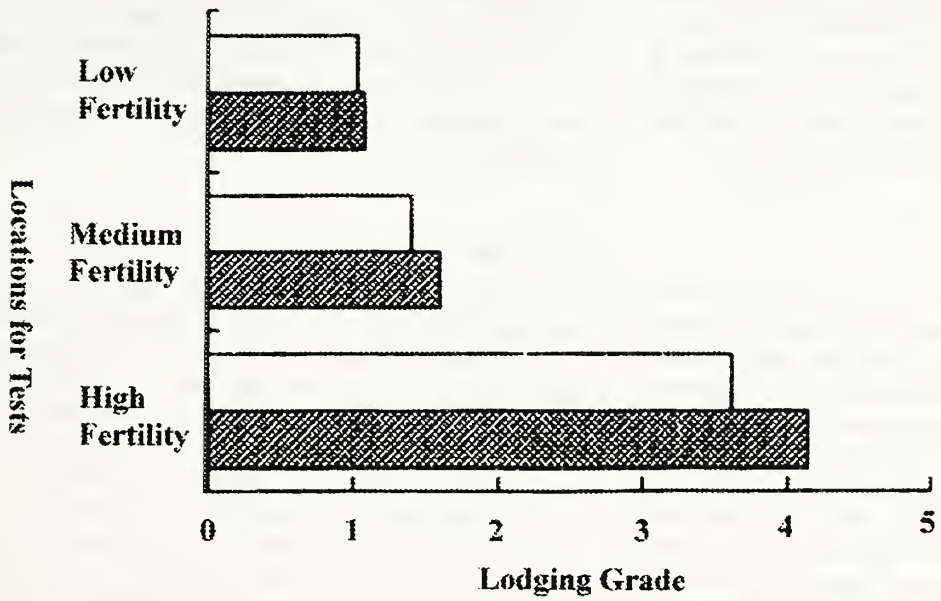

Figure 3. Difference of lodging-resistance between lines selected from high and low fertility

Lodging grade is divided into five grade
grade 1 = all erect, grade 5 = all lodging

□ = Lines selected from high fertility

▨ = Lines selected from low fertility

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Genotypical variability for yield and quality in *Glycine max* L. Merrill.

Soybean tops the world production of oilseeds and edible oil right from 3000 B. C. onwards (Hymowitz, 1970). It provides high quality protein food for man at a low price. Soy-meal, which is obtained after extraction of oil, is being preferentially utilized as animal feed all over the world. Evaluation in terms of biological value and net protein utilization has brought out that soy-protein is superior to protein contained in maize, wheat, and rice (Bhatnagar and Joshi, 1996). With balanced composition of amino acids and 85% polyunsaturated fatty acids of total lipids, soybean is considered to be high quality food for human consumption. The genetic improvement for seed yield and quality aspects of soybean has never been as impressive as in the case of cereals. However, it shall be desirable to look simultaneously into both yield and quality of the soybean so that the material developed may be useful for specific purpose.

Materials and Methods

Elite genotypes belonging to early and normal maturity groups evaluated over three years (1994-96) along with respective check varieties were pooled and analyzed to generate information in the following text (Table 1). All these genotypes were tested at 0.4 and 0.6 million plants per hectare utilizing the recommended package of practices at National Research Centre for Soybean, Indore. The seed yield, harvest index and oil and protein contents were reported. Infratec 1225 was utilized for the non destructive assay of seed oil and protein content.

Results and Discussion

Seed yield

The genotypes in both maturity groups differed significantly with respect to seed yield. From early maturity group, the genotypes PK 1093 (2431 Kg/ha), MACS 390 (2303 Kg/ha), NRC 14 (2213 Kg/ha), SL 231 (2188 Kg/ha), and NRC 7 (2013 Kg/ha) significantly out yielded highest yielding check variety JS 71-05 (1585 Kg/ha). When mean values over two populations were considered, NRC 14 at 0.6 million plants/ha exhibited highest yield followed by PK 1093 at 0.4 million.

In normal maturity group, the genotypes PK 1084 (2369 Kg/ha), PK 1093 (2318 Kg/ha) and PK 1092 (2146 Kg/ha) only numerically gave higher yield over highest yielding check variety JS 335 (2141 Kg/ha). PK 1084 at 0.6 million plants/ha yielded maximum (2477 Kg/ha) followed by PK 1093 (2337 Kg/ha).

In general, the genotypes yielded higher at 0.6 million plants/ha than 0.4 million plants/ha; the mean values for the two population differed significantly. Similar genotypical differences have been reported by Singh et al. (1993) and Tiwari et al. (1994).

Harvest index

The harvest index (HI) was not influenced by maturity groups, plant population, or their interactions (Table 1). Genotypes differed significantly among themselves. Genotypes under early maturity group had relatively higher HI than those under normal maturity group. Among the genotypes, NRC 7, JS 88-13, JS 335, and JS 71-05 showed HI of above 50%. While genotypes namely JS 89-88 and MAUS 53-2 recorded HI less than 30%. Remaining genotypes were intermediate.

Oil content

Genotypes under early maturity group contained comparatively higher oil content than those in under normal maturity group (Table 2). Among genotypes with early maturity, the highest oil content was exhibited by NRC 7 (19.78%). In the rest of the genotypes the oil content ranged from 12.54% (Pb 1) and 18.15% (JS 88-40). In case of genotypes with normal maturity, the maximum oil content was in MAUS 49-1 (17.71%) followed by PK 1095 (17.42%), PK 1092 (17.27%), PK 1084 (17.22%), and PK 1060 (17.07). Remaining genotypes showed oil content ranging between 12.76% (PK 472) and 16.97% (PK 1093).

Protein content

Genotypes with normal maturity showed higher protein content than those in early maturity group, probably owing to the strong negative relationship between oil and protein content (Table 2.) Increased

plant population showed higher protein contents. JS 89-27 possessed the highest protein content (43.37%) followed by SI 231 (41.57%) and MACS 390 (41.05%). The rest of the genotypes with early maturity had protein content less than 40%.

In case of genotypes with normal maturity, PK 1092 (44.07%) showed highest protein content followed by PK 1093 (41.25%), JS 88-66 (41.09%), PK 1084 (41.07%), and PK 1109 (40.12%). The lowest protein was noted in MAUS 431 (37.92%). Genotypes x plant population interaction was found significant. These results also confirm the findings of Singh et. al. (1993) and Prasad et. al. (1993).

Conclusion

The genotypes in early maturity groups were higher in harvest index and oil content, while genotypes in normal maturity group were higher in seed yield and protein content. The genotypes planted at 0.6 million plants/ha had an edge in respect to seed yield, HI, oil content, and protein content than those planted at 0.4 million/ha. Genotypes PK 1093, MACS 390, NRC 14, SL 231, and NRC 7 in early maturity and PK 1084, PK 1093, PK 1092, and JS 335 in normal maturity group were found to be more productive (above 2 t/ha). Genotypes like NRC 7, JS 88-40, JS 88-13, and JS 71-05 in early group; MAUS 49-1, PK 1095, PK 1092, PK 1084, and PK 1060 in normal group were superior for oil production. While for protein production the genotypes JS 89-27, MACS 390 and SL 231, PB 1 and PK 1093 in early group and PK 1092, PK 1093, JS 88-66, PK 1084 and PK 1109 were better. The material above may be put to use in breeding programs for further crop improvement.

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Table 1. Genotypical variation of yield and harvest index of soybean under different planting density

Variety	Yield Kg/ha			Harvest Index Percent		
	Plant population m/ha					
	0.4	0.6	mean	0.4	0.6	mean
Early Maturity Group						
NRC-12	2078	1612	1845	44.40	43.37	43.88
JS 88-40	1742	1854	1798	45.51-	41.59	43.55
JS88-13	1537	1746	1641	55.56	53.95	54.75
PK-1068	1372	1694	1553	47.07	50.50	48.78
MAUS-1.	1004	946	975	43.89	45.39	44.64
NRC-7	1884	2143	2013	57.09	59.97	58.53
JS 71-05 (C)	1522	1649	1585	50.66	50.13	50.39
PB-1 (C)	1313	1392	1352	38.54	40.05	39.29
NRC-14	2076	2449	2213	38.27	38.50	38.38
JS 89-27	1557	1824	1690	40.25	41.09	40.87
MACS-390	2303	2302	2303	41.63	43.67	42.65
SL-231	2152	2218	2188	45.19	43.07	44.13
PK-1093	2440	2423	2431	43.39	43.10	43.24
UGM-52	1465	1350	1408	41.38	33.23	37.30
TAS-41	949	1068	1009	31.38	30.73	31.05
JS 89-48	1059	1184	1122	34.50	35.12	34.81
JS 89-38	738	1150	944	26.76	28.92	27.84
MAUS-38	1432	1058	1245	46.26	35.18	40.72
Monetta (C)	763	1012	888	37.12	43.77	40.44
mean	**1547**	**1630**	**1589**	**42.56**	**42.20**	**42.38**
Normal Maturity Group						
JS 88-66	1566	1486	1706	45.33	43.20	44.26
PK-1060	1054	1295	1174	48.05	48.78	48.42
PK-1095	1576	1599	1587	43.68	43.17	43.42
JS 87-59	2018	2169	2094	48.74	50.17	49.46
JS 335 (C)	2134	2147	2141	54.20	51.75	52.97
JS 80-21 (C)	1134	1152	1143	32.99	31.01	32.00
PK-472 (C)	1314	1580	1447	37.49	40.07	38.78
PK-1084	2260	2477	2369	44.57	44.03	44.30
PK-1092	2036	2255	2146	41.81	42.70	42.25
PK-1093	2299	2337	2318	42.15	49.35	45.75
PK-1109	1743	1815	1779	34.85	35.75	35.30
MAUS 53-2	1034	1039	1037	30.24	28.07	29.15
MAUS 49-1	1169	1285	1227	34.37	33.31	33.83
MACS-431	1210	1360	1285	31.49	33.80	32.65
Mean	**1610**	**1740**	**1675**	**40.70**	**41.08**	**40.89**
Mean	**1574**	**1677**	**1625**	**41.78**	**41.72**	**41.75**

CD(P=0.05)		
Variety	=285	6.17
Plant population	=71	NS
Maturity group	=NS	NS
Variety X pl. population	=403	NS
Maturity group X pl. population	=NS	NS
CV (%)	=15	13.00

Table 2. Genotypical variation of oil and protein content of soybean under different planting density

Variety	Oil percent			Protein percent		
	Plant population m/ha					
	0.4	0.6	mean	0.4	0.6	mean
Early Maturity Group						
NRC-12	18.30	17.35	17.83	35.14	36.75	35.94
JS 88-40	18.23	18.08	18.15	36.66	34.53	37.09
JS88-13	17.99	17.13	18.06	37.71	37.09	37.40
PK-1068	17.05	17.13	17.09	38.14	38.34	38.24
MAUS-1	16.72	17.56	17.14	37.71	16.43	37.07
NRC-7	19.83	19.73	19.78	34.33	35.23	34.78
JS 71-05 (C)	18.19	17.93	18.06	37.60	37.34	37.47
Pb-1 (C)	12.76	12.32	12.54	40.98	42.09	41.53
NRC-14	18.40	17.90	18.15	38.63	39.17	38.90
JS 89-27	17.20	18.67	17.93	43.07	43.67	43.37
MACS-390	15.00	15.00	15.00	40.43	41.67	41.05
SL-231	16.13	15.90	16.02	42.03	41.10	41.57
PK - 1093	17.27	17.03	17.15	40.63	41.03	40.83
UGM-52	16.80	16.87	16.83	36.90	37.80	37.35
TAS-41	15.97	16.47	16.22	37.43	37.30	37.37
JS 89-48	16.60	17.37	16.98	36.37	36.43	36.40
JS 89-38	17.07	16.73	16.90	36.43	37.00	36.72
MAUS-38	16.60	15.67	16.13	36.90	38.73	37.82
Monetta (C)	14.53	15.83	15.18	37.67	38.40	38.03
Mean	**16.88**	**16.93**	**16.90**	**38.15**	**38.59**	**38.36**
Normal Maturity Group						
JS 88-66	13.16	13.74	13.45	48.19	40.99	41.09
PK-1060	17.00	17.14	17.07	37.24	37.36	37.30
PK-1095	17.49	17.35	17.42	37.50	37.71	37.60
JS 87-59	15.20	16.11	15.65	37.48	37.49	37.49
JS 335 (C)	15.70	15.56	15.63	38.02	38.05	38.03
JS 80-21 (C)	15.15	15.25	15.20	38.31	38.18	38.25
PK-472 (C)	12.60	12.91	12.76	40.44	40.31-	40.37
PK-1084	17.23	17.20	17.22	41.13	41.00	41.07
PK-1092	17.33	17.20	17.27	42.17	42.43	42.30
PK-1093	16.93	17.00	16.97	41.33	41.13	41.23
PK-1109	17.10	16.90	17.00	40.10	40.13	40.12
MAUS 53-2	16.17	16.53	-16.35	36.63	36.70	36.67
MAUS 49-1	17.77	17.63	17.70	35.37	35.23	35.30
MACS-431	14.30	-13.93	14.12	37.70	38.13	37.92
Mean	**15.94**	**16.03**	**15.98**	**38.90**	**38.92**	**38.91**
Mean	**16.48**	**16.55**	**16.52**	**38.47**	**38.73**	**38.60**

CD (P=0.05)		
Variety	= 0.50	1.47
Plant population	= NS	0.36
Maturity group	= 0.09	0.25
Variety X Pl. population	= NS	2.08
Maturity group X Pl. population	= NS	NS
CV (%)	= 2.65	3.34

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Stability analysis for oil and yield in soybean

Soybean [*Glycine max* (L.) Merrill] is becoming an important oilseed crop in India covering an area of about 4.7 million hectares with a productivity of about one ton/ha which is very low as compared to Asian average (1.356 ton/ha) and world average (1.913 ton/ha). Soybean is valued for its edible oil (18-20%) and protein rich de-oiled cake fetching 15000 million rupees of foreign exchange.

Soybean crop is generally grown in rainy/normal (June to October) season. However, it can also be grown in post rainy (November to March) and summer (February to May) seasons. Therefore, stability analysis of soybean varieties under different seasons has obvious importance. Few attempts have been made earlier to study the stability of soybean varieties for different seasons/environments/locations (Sharma et al., 1980; Singh and Chaudhary, 1984; Patil et al., 1988; Bhatnagar and Tiwari, 1989; Raut et al., 1990). Hence, the present studies have been undertaken to evaluate yield, oil content and other characters of ten promising soybean varieties during normal season and summer season for stability.

Materials and Methods

The experimental material consisted of ten soybean varieties grown in a randomized block design with four replications under irrigated (E_1) and rainfed (E_2) conditions in normal season and under irrigated conditions in summer (E_3) season during 1991-92 on the Institutes' research farm at Hol (Dist. Pune, Maharashtra State). Each variety was sown in ten rows of 5 m length with 45 cm and 5 cm spacing between and within rows respectively. Sowing dates were 13.7.91, 18.7.91 and 9.2.92. Observations on five random plants were recorded on seven characters: days to flower, days to maturity, plant height (cm), no. of pods/plant, 100 seed weight (g), oil content (%) estimated on NMR, and seed yield on net plot size (4m x 4.5m). Pooled analysis of variance and estimation of stability parameters were carried out by using the model of Eberhart and Russell (1966).

Results and Discussion

Analysis of variance for different characters revealed significant differences between varieties and environments for all the characters (Table 1). Interaction component of variation between varieties and environment was significant only for seed yield. Various parameters of stability for seven quantitative characters are presented in Table 2. Soybean variety MACS-267 was the most stable variety for days to flower. Bragg, JS-335 and Pusa-40 were significantly earlier in flowering whereas only JS-335 was significantly earlier in maturity. MACS-58 and Pusa40 showed more than average stability for days to maturity. MACS-58, Bragg, KHSb-2, and MACS-124 were stable for plant height. MACS-58, KHSb-2, MACS-124, MACS-239, and NRC-2 also showed stability for pods/plant. For 100 seed weight only MACS-267 and Bragg were found to have more than average stability.

Study of seed oil content, an important qualitative character, revealed the highest oil content in MACS-239 (21.06%) followed by Bragg (20.54%) and MACS-58 (20.39%). However, based on regression coefficient (bi) and deviation from regression (s^2d), only MACS-58 and Bragg showed stability for high oil content.

Mean yield performance (X) of ten varieties in three environments indicated JS-335 to be the highest yielder. Likewise, MACS-239, MACS-13, Pusa-40, MACS-124, and MACS-58 were on par with JS-335 for seed yield. Seed yields under rainfed conditions were higher than the irrigated conditions, due to highly fertile soil used for rainfed experiment and even distribution of rainfall during the crop season. Values for regression coefficient were significant for most of the varieties except for NRC-2, Bragg, and MACS-13. However, MACS-13 recorded significant deviation from regression. Considering all the three parameters of stability, JS-335, MACS-239, and MACS-13 are superior among the ten varieties under study.

Mean values for different characters under three environments (Table 3.) indicated rainfed condition (E_2) to be the most favorable followed by irrigated (E_1) and summer (E_3) for seed yield.

However, oil content was highest in E_3 followed by E_1 and E_2. In E_3, there was reduction in plant height and 100 seed weight whereas there was an increase in days to flower and maturity and pods/plant.

Evaluation of soybean varieties for yield, protein, and oil content by Jaipurkar and Thakare (1981) revealed oil percentage as high as 23.7% in EC 9308 and 23.49% in Bragg. Similarly, Khurana and Yadav (1982) could note Bragg, Hill, PK-73-93, and HM-10 soybean varieties to be stable for oil content. In the present studies also in addition to MACS-58, Bragg has recorded more than average stability for oil content. Clark and Snyder (1989) observed significant interaction between years and cultivars for oil content whereas in the present case it appeared to be non-significant.

Sharma et al. (1980) suggested suitable genotypes for rainy and post rainy seasons based on stability analysis. While studying stability of ten soybean varieties sown on different dates, Konwar and Talukdar (1986) found significant genotype x environment interactions for all the characters studied. Sen and Mukherjee (1986) suggested normal season and late (August) as more favorable seasons for soybean cultivation in West Bengal. Improved Pelican was found to be the most stable variety at five locations in Peru (Angeles, 1987). MACS-57 has been reported to be the most stable and high yielding variety for both normal and summer season by Patil et al. (1988) and Raut et al. (1990). Likewise, Bhatnagar and Tiwari (1989) reported MACS-58 as the most stable and high yielding variety in Central India for normal season. These two varieties, MACS-57 and MACS-58 have improved Pelican in their parentage.

Present studies indicated JS-335, MACS-239, and MACS-13 varieties to be stable and high yielding. Likewise, MACS-58 showed high stability for oil content. MACS-239, MACS-58, MACS-124, and JS-335 are more suitable for normal season while JS-335, Pusa-40, and MACS-13 performed well in summer season. Likewise, oil content was observed to be relatively more in summer sowings followed by irrigated and rainfed conditions in normal season.

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Table 1. Analysis of variance for phenotypic stability.

Source	D.F.	Mean squares for						
		Days to flower	Days to mature	Plant height (cm)	100-seed wt. (g)	Pods/plant	Oil content (%)	Seed yield (kg/ha)
1. Variety	9	40.87**	94.88**	761.79**	149.66**	3.66**	3.49**	370490.70**
2. Environment + (Variety x Environment)	20	50.57**	16.04*	70.47	248.83**	1.22	0.15	553807.20**
3. Environment (linear)	1	968.02**	208.02**	875.48**	4005.66**	15.55**	1.44**	9424332.00**
4. Variety x Environment (linear)	9	3.46	6.36	12.54	0.39	59.64	0.10	150293.40*
5. Pooled deviation	10	1.23**	5.56**	42.11**	43.15	0.53**	0.07**	29917.15
6. Pooled error	90	0.41	0.70	13.26	37.51	0.10	0.01	17317.00

*, ** Significant at 5% and 1% respectively.

Table 2. Stability parameters for 7 characters in soybean.

Varieties	Days to flower			Days to mature			Plant height (cm)			Pods/plant			100-seed wt. (g)			Oil content (%)			Seed yield (kg/ha)		
	X	bi	s^2di	X	bi	s^2di	X	bi	s^2di	X	bi	s^2di	X	bi	s^2di	X	bi	s^2di	X	bi	s^2di
MACS-239	42.67	0.91**	0.60	92.17	0.25**	0.11	86.85**	1.18	111.06**	57.53	1.05	76.13	13.08	0.95**	-0.09	21.06**	1.53	0.12**	3049.50	1.17**	-15669.60
MACS-58	42.67	0.91**	0.60	91.67	0.92	1.61	78.53	1.46	24.65	54.88	0.74	22.06	14.67	1.61	0.63**	20.39**	0.90	0.02	2803.67	1.36**	-17244.80
JS-335	38.33	0.84**	-0.16	86.00**	1.31**	-0.19	46.08	0.67**	-13.24	36.20*	0.45**	-37.51	14.03	1.76**	-0.01	19.72	0.53	0.05*	3165.00*	0.22**	-16091.70
NRC-2	40.67	1.17**	-0.39	89.25	0.75	14.57**	40.43	1.13	80.02**	48.22	1.03	-30.40	12.71	0.62	0.31*	18.74	0.02	0.05*	2712.67	1.02	27187.49
MACS-267	44.50	1.00	-0.25	91.17	0.74**	0.04	79.82**	1.70**	-11.92	57.76	1.82**	-21.93	13.12	1.08	0.04	18.61	2.60**	0.02	2255.17	1.43**	-17316.50
Pusa-40	39.33	0.84**	-0.16	95.08	0.73	2.36*	68.20	0.74	101.44**	44.88	0.95*	-32.13	15.07	1.30	0.50*	19.52	1.85*	0.02	2947.25	0.55**	6515.59
Bragg	36.67	1.02	2.98**	90.50	0.87	4.96**	48.12	0.64	-0.21	44.80	0.53**	8.37	14.75	0.64	0.23	20.54**	-0.01*	0.03	2565.17	0.92	23835.94
KHsb-2	49.67**	1.42**	-0.39	104.00**	2.16	14.45**	73.40	1.10	-6.02	54.05	1.09	104.10	15.40	0.76	1.43**	20.05*	0.30	1.10**	2088.00**	1.47**	-13472.10
MACS-124	45.83**	0.80*	4.98**	93.00	0.62	5.24**	72.85	0.57	15.70	55.68	1.06	5.17	15.83*	1.20	0.95**	17.95	0.81	0.10**	2911.42	1.06**	-12263.90
MACS-13	46.83**	1.10**	0.45	102.33**	1.62	5.42**	58.37	0.81**	-13.00	54.45	1.27**	-37.48	15.35	0.07**	0.27	18.06	1.47*	0.01	3001.08	0.80	160521.10**
Mean	42.72			93.52			65.35			50.85			14.40			19.47			2749.89		
CD(P=0.05)	2.47			5.25			14.46			14.64			1.62			0.58			385.37		
r(X, bi)		0.50			0.65			0.50			0.71*			-0.06			-0.22			-0.71*	
r(X, s^2di)		0.12			0.43			0.18			0.46			0.68*			0.25			0.23	
r(bi, s^2di)		-0.39			0.47			0.04			-0.02			-0.03			-0.25			-0.24	

*, ** Significant at 5% and 1% level, respectively.

Table 3. Mean performance of 10 soybean varieties and environmental indices for 7 characters in different environments.

Varieties	Days to flower			Days to mature			Plant height (cm)			Pods/plant			100-seed Wt. (g)			Oil content (%)			Seed yield (kg/ha)		
	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3	E_1	E_2	E_3
MACS-239	39	39	50	93	91	93	95	91	74	51	45	77	13.2	13.9	12.2	20.78	20.78	21.62	3202	3764	2182
MACS-58	39	39	50	89	91	95	90	69	73	42	58	65	14.3	16.4	13.3	20.55	20.07	20.54	3013	3618	1780
JS-335	36	34	45	85	83	91	51	44	46	31	34	43	14.2	15.5	12.4	19.93	19.48	19.75	3228	3275	2992
NRC-2	37	35	50	85	90	93	49	44	29	38	42	65	12.3	13.5	12.3	18.96	18.64	18.64	3042	3201	1895
MACS-267	41	40	53	90	90	94	93	75	72	39	48	87	13.7	13.7	12.0	18.48	17.98	19.38	2482	3103	1180
Pusa-40	37	35	46	93	95	98	75	58	72	35	39	60	14.7	16.5	14.0	19.69	18.94	19.93	3158	3190	2495
Bragg	32	33	45	88	90	94	54	49	44	35	48	52	14.4	15.6	14.3	20.72	20.47	20.45	2874	3003	1819
Khsb-2	45	43	61	104	97	112	82	68	71	34	59	69	16.6	15.3	14.4	19.78	20.10	20.28	2272	2997	996
MACS-124	45	41	52	94	90	95	78	67	74	47	46	74	16.9	16.2	14.4	17.69	17.86	18.31	3022	3578	2134
MACS-13	44	41	56	102	97	108	65	55	55	40	49	74	15.9	15.1	15.1	18.20	17.61	18.39	2791	3704	2509
lj	-3.22	-4.77	7.98	-1.37	-2.32	3.68	7.62	-3.34	-4.29	-11.78	-3.92	15.70	0.21	0.76	-0.97	0.01	-0.27	0.26	158.43	593.38	-751.82
Mean	39.5	38.0	50.7	92.2	91.2	97.2	73.0	62.0	61.1	39.1	46.9	66.5	14.61	15.16	13.43	19.48	19.19	19.73	2908	3343	1998
CD(P=0.05)	0.00	0.78	3.61	2.30	2.12	3.72	13.70	8.88	13.42	14.26	22.13	23.89	0.66	0.25	0.91	0.37	0.35	0.50	334	395	562

E_1 - Normal season (irrigated)
E_2 - Normal season (rainfed)
E_3 - Summer (irrigated)

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Variability, correlation and path analysis in soybean hybrids

Soybean [*Glycine max* (L.) Merrill] is grown on an area of about 4.7 million hectares in India. Due to rapid development of processing industries, farmers are inclined to grow this crop. In order to increase productivity it is necessary to develop high yielding varieties. Variability, correlation, and path analysis studies furnish background information for improvement in quantitative characters. Earlier reports indicate that such studies have been made by using cultivars. However, such studies are meager in F_1 hybrids. Hence, present studies have been undertaken to study the role of quantitative characters through variability, correlation, and path analysis in order to select better varieties.

Materials and Methods

Crosses were attempted to get F_1's between six promising cultures: MACS-13, MACS-111, Monetta, Gaurav, Improved Pelican, and Nimsoy-7'. Nine cross combinations along with parents were planted in *kharif* season of 1987 in a randomized block design with three replications. Row length was 3 m and a distance of 45 cm and 15 cm was kept between and within rows. Data were recorded on 5 randomly selected plants on eight quantitative characters and processed further to work out variability parameters, correlation, and path coefficients as per standard procedures (Singh and Choudhary, 1979).

Results and Discussion

Analysis of variance indicated significant differences among parents and hybrids for all the characters. Range, mean, and standard error revealed a high amount of phenotypic variability for all characters (Table 1).

Genotypic coefficient of variation (GCV) was highest for plant height followed by pods/plant and seeds/plant indicating importance of these characters in selection. Iglesias (1988) reported plant height as the most important character in selection. In general, values of Phenotypic Coefficient of Variation (PCV) were higher than GCV. Heritability estimates (in broad-sense) were highest for days to maturity followed by plant height and days to flower. For other characters moderate to high values of heritability were observed. Alam et al. (1983) reported high heritability estimates for days to flower, total growth period, plant height, seeds/pod, and oil and protein content. Similarly, Yao et al. (1987) recorded high heritability estimates for most of the yield components. High heritabilities are always preferred since selection can be reliably based on phenotypic expression of such characters.

In conjunction with GCV and heritability, expected genetic gain should be considered while selecting particular characters (Panse, 1957). In the present studies, plant height, pods/plant, seeds/plant, and 100-seed weight showed high heritability estimates with high expected genetic gain, which indicated that these characters are mainly governed by additive gene effects and improvement can be made by selecting for these characters.

Correlation studies (Table 2) indicated that the values of genotypic correlation coefficients were higher than their respective phenotypic correlation coefficients. Significant positive correlation was observed between seed yield/plant with plant height, branches/plant, pods/plant, and seeds/plant. 100-seed weight showed significant negative correlation with other characters. However, 100-seed weight recorded weak negative correlation with seed yield/plant. Significant positive correlations were observed among all combinations of days to flower, days to mature, plant height, branches/plant, and seeds/plant. Present studies are in corroboration with those of Alam et al. (1983), Yao et al. (1987), and Bargale et al. (1988b).

Direct and indirect effects of different characters on seed yield/plant are presented in Table 3. The highest direct effect was recorded by seeds/plant followed by 100-seed weight, days to flower, branches/plant, and pods/plant. Though there was negative correlation between 100-seed weight and seed yield/plant, 100-seed weight had high positive direct effect on seed yield. This is attributed to the highly negative indirect effects via seeds/plant and days to flower. Seeds/plant exerted highly positive indirect effects, whereas 100-seed weight showed highly negative indirect effects via the other

characters. Thus, the path analysis studies revealed importance of seeds/plant, 100-seed weight and branches/plant in selecting better genotypes.

Wakankar et al. (1974) reported branches/plant, flowering cluster number, and pod number as important characters. Plant height and pod number are noted as main yield components by Choudhari and Singh (1974) and Sharma (1979). High direct positive effects of pods/plant, seeds/pod, and 100-seed weight on seed yield/plant have been reported by Bargale et al. (1988a).

Hence, based on variability, correlation, and path analysis studies it can be concluded that better genotypes can be selected in advanced segregating generations on the basis of phenotypic expression of branches/plant, seeds/plant, and 100-seed weight.

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Table 1. Phenotypic and genotypic variability in soybean

Character	Range	Mean	PCV	GCV	Heritability	Genetic advance	Genetic gain
Days to flower	37.00-47.00	43.27	8.51	7.47	77.05	5.85	13.52
Days to mature	79.00-95.00	92.13	4.58	4.44	94.05	8.17	8.87
Plant height (cm)	14.30-61.33	38.94	38.80	34.54	79.22	24.66	63.33
Branches/plant	3.50-7.33	5.16	24.11	19.11	62.80	1.60	31.10
Pods/plant	38.87-164.40	99.51	44.71	34.28	58.79	53.88	54.15
Seeds/plant	93.00-286.20	170.78	41.65	31.63	57.67	84.51	49.48
100-seed weight (g)	8.50-13.07	10.60	33.63	25.44	57.22	7.03	66.31
Seed yield/plant (g)	12.17-25.77	17.73	15.85	12.76	64.44	2.25	12.66

Table 2. Phenotypic (upper diagonal and genotypic lower diagonal correlation) coefficients between yield components in soybean.

Character	Days to flower	Days to mature	Plant height (cm)	Branches/ plant	Pods/ plant	Seeds/ plant	100-seed weight (g)	Seed yield plant (g)
Days to flower	-	0.73**	0.55**	0.24	0.39*	0.24	-0.65**	0.03
Days to mature	0.85**	-	0.60**	0.39*	0.44*	0.34	-0.42*	0.26
Plant height (cm)	0.79**	0.64**	-	0.37*	0.57**	0.53**	-0.60**	0.40*
Branches/plant	0.44*	0.48**	0.46*	-	0.71**	0.61**	-0.34	0.63**
Pods/plant	0.53**	0.52**	0.80**	0.92**	-	0.84**	-0.48**	0.77**
Seeds/plant	0.40*	0.40*	0.90**	0.92**	1.00**	-	-0.50**	0.90**
100-seed weight (g)	-0.92**	-0.60**	-0.99**	-0.52**	-0.74**	-0.65**	-	-0.16
Seed yield/plant (g)	0.08	0.24	0.57**	0.95**	0.90**	0.94**	-0.36	-

*, ** Significant at 5% and 1% respectively

Table 3. Direct (underlined) and indirect effects of yield components on seed yield in soybean.

Characters	Days to flower	Days to mature	Plant height (cm)	Branches/ plant	Pods/ plant	Seeds/ plant	100-seed weight (g)	Correlation with seed yield plant (g)
Days to flower	0.39	-0.19	-0.01	0.08	0.06	0.43	-0.68	0.08
Days to mature	0.34	-0.23	-0.01	0.09	0.06	0.43	-0.44	0.24
Plant height (cm)	0.31	-0.15	-0.01	0.08	0.10	0.97	-0.73	0.57**
Branches/plant	0.17	-0.11	-0.01	0.18	0.11	0.99	-0.39	0.95**
Pods/plant	0.21	-0.12	-0.01	0.17	0.12	1.08	-0.55	0.90**
Seeds/plant	0.16	-0.09	-0.01	0.17	0.12	1.08	-0.48	0.94**
100-seed weight (g)	-0.36	0.14	0.01	-0.09	-0.09	-0.70	0.74	-0.36

*, ** Significant at 5% and 1%, respectively

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Variation in Sensitivity of Soybean Genotypes to Varying Photoperiods in India*

Introduction

During the last two decades, fifty varieties of soybean have been identified/released in India for cultivation (Bhatnagar and Karmakar, 1995). The range of adaptation of these varieties is reported to be limited (Taware et al., 1991, Bhatnagar and Karmakar, 1993). Soybean is a short day plant which was first recognized by Garner and Allard (1920). In India, soybean is mainly a rainy season crop and its planting depends largely on the onset of the monsoon which varies from year to year. Consequently, the yield performance exhibited by the crop invariably fluctuates depending on the planting time. Earlier studies indicate that soybean varieties differ in their responses for flowering to photoperiods (Johnson et al., 1960; Byth, 1968). In view of the above, there is a need to generate information on the photoperiodic behavior of soybean varieties in India. Hence, the present study was undertaken to screen the soybean varieties for their photoperiodic requirements with respect to flowering and other reproductive stages. A knowledge of photoperiodic requirement of these varieties would be of value in determining possible time of planting at a given location and areas of their adaptation.

Materials and Methods

Thirteen soybean varieties and two promising test cultivars (NRC7 & NRC1) were sown in pots in the month of August 1995 at National Research Centre for Soybean, Indore. The minimum and maximum temperature ranged from 11.1 to 22.7°C and 28.2 to 33.8°C, respectively from August to December when the experiment was terminated.

All the varieties/genotypes were grown under natural and 9 photoperiods at an hourly difference starting from 7 to 15 hours. For the shorter photoperiods, pots were shifted to dark polyhouses for their dark hours. The photoperiods exceeding the natural were achieved by the use of artificial incandescent lights. The precise photoperiod treatments were achieved by using separate timer clocks wired to a series of 40 Watt incandescent bulbs placed above the pots at a fixed height. The pots were arranged in a circle and the plants received a uniform light intensity of 0.25 mW/cm^2 measured at the top of the canopy with a Radiometer (IL 1350, International Light Inc., USA). This light intensity is adequate to elicit a photo response in the varieties of soybean as reported earlier (Yoshida, 1952). The photoperiod treatments were started at the emergence of the first trifoliate leaf. Photoperiodic behavior of the following variables were recorded: Days from planting until opening of first flower (R1), days from R1 to beginning pod (R3), days from R3 to beginning seed (R5), days from R5 to full seed (R6), and days from R6 to physiological maturity (R7 stage).

The experiment was terminated after 128 days. A factorial design was used to analyze photoperiod treatment and genotype effects for R1 growth stage. For other characters, simple one way analysis of variance was carried out separately for photoperiods and genotypes, respectively.

Results and Discussion

There were no significant differences in days to flowering and all other characters in photoperiods ranging from 7 to 10 hours. The data for 8, 9, and 10 hours of photoperiods are not presented here.

Days to Flowering

There were significant differences in days to flowering with respect to photoperiod beyond 10 hours (Table 1). The mean days to flowering under natural photoperiod was 36 days. Although the values for days to flowering showed a decreasing trend, the numerical differences were marginal in 7 to 11 hours of photoperiods (33 days). On extending the photoperiods beyond 11 hours the days to flowering started exhibiting an increasing trend and the maximum increase was observed in 14 (47 days) and 15 hours (71 days) of photoperiods. There were significant differences among the 15 genotypes for their days to flowering. The minimum time to flowering was taken by JS 71-05 (32 days) followed by NRC 7 (35 days). The maximum time was 49 days

* An Inter-institutional joint study at NRCS, Indore

recorded for Hardee. The photoperiod x genotype interaction was also found to be significant indicating a differential response of these varieties to varying photoperiods. The minimum variation was exhibited by Hardee (35 to 95 days).

Pod Initiation

Among the 15 genotypes, only Hardee did not reach to pod initiation stage in the 15 hour photoperiod. There were significant differences in days to pod initiation from flowering among the various photoperiods and genotypes (Table 2). The trend in pod initiation with respect to individual genotypes and their mean revealed no perceptible difference up to 11 hours of photoperiod. Some of the varieties like Monetta and Pb 1 showed the least variation in days to pod initiation. It is interesting to note that JS 71-05 and NRC 7 which were least affected by varying photoperiods for onset of flowering were found to take maximum time for pod initiation in 15 hour photoperiod.

Seed Initiation

Out of 15 genotypes, 6 and 8 did not reach seed initiation stage in 14 and 15 hours of photoperiod, respectively (Table 3). A trend similar to occurrence of flowering and pod initiation was observed in genotypes up to 14 hours of photoperiods.

Full seed and physiological maturity

None of the genotypes reached full seed stage and physiological maturity in 14 and 15 hours while in 13 hours of photoperiod only MACS 124 did not reach these stages. Unlike earlier stages, there was no definite trend in these stages.

Conclusion

The data presented indicated that the initiation of flowering and beginning pod stages of the reproductive phase in soybean were most susceptible to variation in photoperiods. Of the fifteen genotypes screened under varying photoperiods, the early maturing ones (JS 71-05, NRC 7, NRC 2 and Monetta) seem to be less sensitive to photoperiods as compared to the late maturing ones (PK 472, Hardee, MACS 124 and Co 1). The variety JS 71-05 to some extent appears to be non-sensitive to photoperiod with respect to initiation of flowering. Extension of photoperiod did not prevent flowering in any of the genotypes but delayed the same. In the longer photoperiods (14 and 15 hours) though flowering occurred, normal pod development did not take place and the pods did not reach R6 and R7 stages of development. The photoperiodic suitability of any soybean variety needs to be analyzed at all stages of pod development. Inspite floral induction, the later stages of pod development are not normal under longer photoperiods. The findings reported in this paper are preliminary but confirm the importance of this study.

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Table 1. Effect of various photoperiods on days to flowering (R1) in 15 Indian soybean genotypes.

Genotypes	Photoperiods (hrs.)							
	Natural	7	11	12	13	14	15	Mean
JS 71-05	30.0	30.3	28.0	30.3	30.6	34.0	37.3	31.5
NRC 7	32.0	32.3	30.7	32.7	31.7	37.3	50.7	35.3
NRC 2	33.3	31.3	32.0	34.7	35.7	45.7	59.7	38.9
Monetta	34.7	32.0	32.3	34.7	35.0	42.0	77.7	41.2
Bragg	34.7	32.7	32.7	33.0	37.0	47.7	77.0	42.1
PK 564	34.7	35.3	32.7	36.0	36.0	45.7	77.0	42.5
PK 416	35.3	33.3	32.7	36.3	37.0	45.7	60.7	40.1
JS 335	35.3	32.0	35.3	36.3	37.3	45.0	59.3	40.1
Pb 1	36.0	33.0	32.7	40.0	41.7	47.3	83.3	44.9
PK 262	38.3	35.3	35.3	39.3	41.3	49.3	77.3	45.2
NRC 1	38.7	32.7	32.3	36.7	42.0	50.0	77.0	44.2
PK 472	38.7	35.7	35.0	37.7	41.7	51.7	81.3	45.9
Hardee	39.7	35.3	35.0	43.7	43.3	49.7	95.3	48.9
MACS 124	40.0	35.7	35.3	40.0	42.7	52.7	77.3	46.2
Co 1	42.3	35.0	34.7	43.0	43.0	54.3	77.0	47.1
Mean	36.3	33.5	33.1	36.9	38.4	46.5	71.2	42.3

LSD (0.05 p)
Photoperiods (P) 0.28
Genotypes (G) 0.40
PXG 1.07

Table 2. Effect of various photoperiods on days from flowering (R1) to beginning pod (R3) stage in 15 Indian soybean genotypes.

Genotype	Photoperiods (hrs)								
	Natural	7	11	12	13	14	15	Mean	LSD#
JS 71-05	4.0	3.0	3.3	3.7	6.3	6.0	24.0	7.2	2.09
NRC 7	4.3	3.3	4.0	4.0	5.7	6.3	27.3	7.9	1.71
NRC 2	4.7	3.7	3.0	3.3	7.3	9.3	18.3	7.1	0.94
Monetta	3.3	2.3	3.0	4.0	4.0	8.7	7.7	4.7	1.01
Bragg	4.3	5.7	3.3	4.7	7.0	8.7	12.7	6.6	1.87
PK 564	4.3	3.3	3.3	3.0	5.0	7.7	7.3	4.9	1.58
PK 416	3.7	5.0	2.7	2.7	5.0	8.7	14.0	5.9	1.53
JS 335	3.0	4.7	3.7	3.7	6.7	9.3	18.0	7.0	1.08
Pb 1	6.3	3.3	3.0	3.3	5.0	10.0	5.0	5.1	0.94
PK 262	3.3	4.3	3.7	4.0	5.7	6.0	10.0	5.3	1.21
NRC 1	6.0	3.0	5.3	7.3	4.7	9.0	13.3	6.9	1.67
PK 472	3.3	4.7	2.7	6.0	4.3	11.0	9.7	5.9	1.58
Hardee	4.0	3.0	3.0	6.0	9.0	15.0	*	6.8	1.11
MACS 124	5.7	3.7	2.7	5.0	8.7	11.3	14.7	7.4	0.94
Co 1	8.0	4.3	4.3	7.3	8.3	10.3	12.7	7.9	0.94
Mean	4.5	3.8	3.4	4.5	6.8	9.2	13.9	6.5	
LSD#	0.93	1.27	0.99	1.02	0.86	2.79	1.90		

Table 3. Effect of various photoperiods on days from beginning pod (R3) to seed initiation (R5) stage in 15 Indian soybean genotypes.

Genotype	Photoperiods (hrs)								
	Natural	7	11	12	13	14	15	Mean	LSD#
JS 71-05	12.0	7.3	7.3	10.0	12.3	20.3	33.7	13.3	1.83
NRC 7	9.7	5.3	7.7	6.0	11.7	19.3	11.3	10.1	1.32
NRC 2	15.0	10.7	9.7	9.3	18.3	25.3	8.3	13.8	1.48
Monetta	15.3	8.0	7.0	5.7	9.3	35.0	12.3	13.4	1.53
Bragg	8.3	7.3	8.3	12.0	10.7	*	*	9.3	1.88
PK 564	8.7	7.7	9.7	10.0	11.7	24.3	14.0	12.3	1.71
PK 416	11.7	8.0	8.3	9.0	12.0	23.3	18.7	13.0	1.53
JS 335	9.3	10.0	8.3	9.7	20.3	30.7	15.3	14.8	1.62
Pb 1	9.0	10.3	9.7	13.3	15.3	23.3	*	13.5	2.01
PK 262	10.3	8.0	8.7	12.3	15.0	*	*	10.9	1.15
NRC 1	10.0	10.7	9.7	12.7	17.7	*	*	12.1	2.49
PK 472	12.0	10.7	9.3	10.7	18.0	31.7	*	15.4	2.94
Hardee	16.3	8.3	8.3	10.7	17.3	*	*	12.2	1.56
MACS 124	8.7	8.0	8.7	12.7	13.3	*	*	10.3	1.69
Co 1	12.3	8.7	7.3	13.7	14.7	*	*	11.3	1.76
Mean	11.36	8.56	8.53	10.51	15.27	25.93	14.81		
LSD#	1.74	2.35	1.02	1.08	1.34	1.87	2.20		

Table 4. Effect of various photoperiods on days from seed initiation (R5) to full seed (R6) stage in 15 Indian soybean genotypes.

Genotype	Photoperiods (hrs)								
	Natural	7	11	12	13	14	15	Mean	LSD#
JS 71-05	8.3	16.7	12.7	12.0	11.3	*	*	12.2	1.49
NRC 7	7.0	20.3	10.3	16.7	11.7	*	*	13.2	1.49
NRC 2	8.7	15.7	9.7	12.7	9.0	*	*	11.1	1.88
Monetta	7.3	14.0	10.7	16.3	11.7	*	*	12.0	1.69
Bragg	10.0	15.7	12.3	12.0	21.7	*	*	14.3	1.99
PK 564	11.3	16.0	9.0	11.0	11.3	*	*	11.7	1.05
PK 416	7.7	14.3	10.3	12.7	13.3	*	*	11.7	1.76
JS 335	10.7	14.0	7.0	11.0	20.0	*	*	12.5	1.69
Pb 1	10.3	14.0	9.3	12.3	16.0	*	*	12.4	1.63
PK 262	9.3	17.7	13.0	20.7	15.0	*	*	15.1	1.82
NRC 1	9.3	16.0	8.3	10.0	20.7	*	*	12.9	3.04
PK 472	9.3	14.7	9.3	9.3	12.7	*	*	11.1	2.53
Hardee	10.7	15.7	12.0	18.0	14.0	*	*	14.1	1.33
MACS 124	15.0	14.7	9.0	8.0	*	*	*	11.7	1.72
Co 1	8.0	16.7	13.0	11.0	19.7	*	*	13.7	2.66
Mean	9.6	15.8	10.9	12.9	14.9	*	*	12.7	
LSD#	1.92	2.16	1.45	1.24	1.84				

Table 5. Effect of various photoperiods on days from full seed (R6) to physiological maturity (R7) stage in 15 Indian soybean genotypes.

Genotype	Photoperiods (hrs)								
	Natural	7	11	12	13	14	15	Mean	LSD#
JS 71-05	13.7	16.0	13.7	14.0	16.3	*	*	14.7	1.49
NRC 7	15.3	14.3	13.0	13.3	15.0	*	*	14.2	1.82
NRC 2	10.3	14.3	14.7	14.3	9.0	*	*	12.5	1.69
Monetta	8.0	9.7	8.3	5.7	15.7	*	*	9.5	2.05
Bragg	12.0	14.3	14.0	14.3	8.3	*	*	12.6	1.82
PK 564	11.7	15.3	12.3	17.0	13.7	*	*	14.0	2.49
PK 416	11.0	16.3	14.3	13.7	10.7	*	*	13.2	1.24
JS 335	9.3	12.3	12.0	14.3	10.0	*	*	11.6	3.55
Pb 1	10.3	12.3	11.3	7.7	7.0	*	*	9.7	2.20
PK 262	13.0	11.3	12.0	7.3	12.0	*	*	11.1	3.20
NRC 1	14.0	15.7	13.7	14.0	12.0	*	*	13.9	2.40
PK 472	13.0	11.3	16.3	16.3	13.7	*	*	13.5	4.10
Hardee	8.0	15.0	18.0	11.3	15.0	*	*	14.1	1.69
MACS 124	6.3	17.3	16.0	11.3	*	*	*	12.7	2.49
Co 1	9.0	14.7	18.0	13.0	12.0	*	*	13.4	2.10
Mean	10.9	14.0	13.8	12.5	12.2	*	*	12.7	
LSD#	2.12	2.96	2.22	1.55	1.86				

Significant at 0.05 p

* Observations not included in statistical analysis because the plants did not reach these growth stages

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Agronomic Evaluation of a Sinuate-Leaf Type Soybean

Introduction

In the summer of 1992, sinuate-leaf plants, plants with a distinctly wavy margin, were identified in $F_{4:5}$ plant rows. Genetic analysis revealed that the sinuate-leaf trait is controlled by two recessive genes. Preliminary information suggested smaller seeds were associated with the sinuate-leaf type. The purpose of this study was to determine if the sinuate-leaf trait affects agronomic performance and seed composition.

Materials and Methods

In 1992 at the University of Illinois Cruse Farm, we found soybean plants with sinuate leaves in $F_{4:5}$ plant rows that originated from the cross [LN84-7513, (selection from (Hack x Elgin)) x Harper 87] (Nickell et al., 1985; Fehr and Bahrenfus, 1984; Fehr et al., 1988).

Homozygous normal and sinuate rows were randomly selected and categorized according to maturity. The maturity Group II test included 5 normal-leaf and 6 sinuate-leaf types, the maturity Group III test included 12 normal leaf lines and 20 sinuate-leaf lines, and a maturity Group IV test contained 27 normal leaf lines and 5 sinuate-leaf lines.

In 1993, each entry was planted in two-row plots at two locations in Illinois (Agronomy-Plant Pathology South Farm and Cruse Farm). In 1994, plot size was increased to four rows with a 76-cm row spacing between rows. In both years a randomized complete block design with two replications was used. For each plot, approximately 150 seeds were planted in each of the 4.5 m rows. At the R-6 growth stage these rows were trimmed to 3 m. Two center rows from each plot were harvested and seed yield was adjusted to 130g kg^{-1} moisture. Eight traits were evaluated: (i) yield (kg/ ha), (ii) maturity (date when at least 95% of the pods had mature color), (iii) lodging (scored based on 1 = all plants erect, 5 = all plants lying flat on the soil surface), (iv) plant height at harvest (cm), (v) seed quality, based on the amount of wrinkled and discolored seed (on a scale of 1 = excellent to 5 = poor), (vi) seed weight (mg), (vii) seed protein (g/kg), and (viii) seed oil (g/kg). A sample for protein and oil determination was collected by combining seed from the two replications at a location. Protein and oil values were measured with near-infrared reflectance at the National Center for Agricultural Research, Peoria, IL (Rinne et al, 1975).

The experimental design was a randomized complete block with two replicates per location combined over two years. In the analysis of variance (ANOVA) the effects of year and location were considered random, whereas leaf type was considered fixed. Protein and oil values were analyzed separately, and in these analyses, locations were considered as replications within each year. Statistical computations were carried out using SAS system for Windows (SAS Release 6.08 for personal computers; SAS Institute, Cary, NC). Significance was determined using a *F*-test as described by McIntosh (1983). Means were separated at the 0.05 probability level using the least significant difference (LSD).

Results and Discussion

There is a year x location effect for every trait evaluated, which implies that there was variation between year and location environments. Specifically, one factor contributing to the interactions was generally a cool and wet growing season in 1993, while 1994 was warmer and drier. Year, location, and leaf-type effects were not significant. It is interesting that the leaf x year interaction was not significant for any of the traits. Since sinuate leaf was apparent in 1993 and not in 1994, we can further conclude that the sinuate-leaf did not affect any of the agronomic traits.

Mean values between normal-leaf entries and sinuate-leaf entries for the maturity Group II study was only significant for maturity (Table 1). This difference can be explained by misclassifying maturity for some sinuate-leaf rows when grouping the single rows according to maturity.

Normal leaf entry mean was significantly higher than the mean of the sinuate-leaf entries for yield in the maturity Group IV (Table 1). However, the mean seed weight for the sinuate-leaf type entries was significantly greater than that of the normal-leaf entries (Table 1).

The ANOVA for protein and oil also showed a year effect for protein in the maturity Group II study. In maturity Groups III and IV, we found an interaction

between year x location. Normal and sinuate-leaf entry means in maturity Groups III and IV were significantly different for oil percentage (Table 1). The sinuate-leaf entries= means were 5 g/kg lower in oil content than the normal-leaf entries. Much of this difference can be attributed to the extremely high oil content of two normal-leaf entries, LN92-4577 and LN92-4608.

Sinuate leaf did not affect any of the agronomic and seed composition traits evaluated.

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Table 1. Comparison of means for agronomic performance of normal and sinuate leaf soybean lines averaged over two years and two locations.

					Seed			
	Yield	Maturity	Height	Lodging	Quality	Weight	Protein	Oil
	kg /ha	date[1]	cm	score[2]	score[2]	mg	---------- g /kg ----------	
Group II								
normal	2819	Sept. 17	84	1.2	2.0	189	412	213
sinuate	2778	Sept. 23	82	1.2	1.9	188	415	212
LSD (0.05)	n.s.	1.9	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Group III								
normal	2868	Sept. 21	92	1.3	1.8	176	414	211
sinuate	2837	Sept. 21	94	1.4	1.9	179	415	206
LSD (0.05)	n.s.	n.s.	1.8	0.1	0.1	n.s.	n.s.	2
Group IV								
normal	2917	Oct. 2	100	1.6	1.6	163	411	204
sinuate	2781	Oct. 3	96	1.5	1.5	170	414	199
LSD (0.05)	127	0.8	3	n.s.	n.s.	6	n.s.	2

[1] Maturity, date when 95% of pods have mature pod color.
[2] Score 1 to 5, where 1 = excellent and 5 = poor.

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Genetic Analysis of a Sinuate-Leaf Type Soybean.

Introduction

A sinuate-leaf type soybean plant derived from non-sinuate leaf parents was first observed in $F_{4:5}$ rows at the University of Illinois Cruse farm in late July of 1992. Our first impression was that the sinuate leaf may be caused by soybean mosaic virus (SMV). However, with the uniformity of the phenotype, we felt the sinuate-leaf type was not a disease. This was later confirmed by Dr. Glen Hartman, USDA Soybean Plant Pathologist at the University of Illinois, after he tested and found no SMV.

The sinuate leaf soybean plants are visible from mid-July until yellowing and dropping of the leaves in the fall. Sinuate leaves first appear at the ninth node of the soybean plant. The leaves are yellowish green with a bumpy surface and a sinuate margin. The expression of sinuate leaves may vary within the population. The objectives of this study are to determine if this trait is heritable, and to determine how many genes are responsible forsinuate leaves.

Materials and Methods

The $F_{4:5}$ plant rows used in this study were derived from the cross [LN84-7513 (a selection from (Hack x Elgin)) x Harper 87] (Fehr and Bahrenfus, 1984; Nickell et al., 1985; Fehr et al., 1988). The 252 plant rows were classified as either normal, heterogeneous, or sinuate leaf on 29 July, 1992 (Table 1). Within the heterogeneous rows, sinuate-leaf plants were tagged and counts were made. The chi-square test was used to measure goodness of fit to expected genetic ratios.

In 1993, progenies fromsinuate-leaf lines were planted next to T176 and T205 [wavy-leaf types (lw_1lw_2)] and L65-701 [bullate-leaf type (lb_1lb_2)] (USDA Soybean Germplasm Collection); all with leaf abnormalities (Palmer andKilen, 1987).

During the summer of 1992, crosses were made at the Agronomy Plant Pathology South Farm, Urbana, IL between LN92-4353 (sinuate-leaf line) and T176, a wavy-leaf line described by Rode and Bernard (1975) to determine if they have different genes controlling leaf type. Successful crosses were confirmed by flower and pubescence color phenotypic markers in the parental lines.

Results and Discussion

When the sinuate-leaf type plants were compared with T176 and T205 (both wavy-leaf types) and L65-701 (bullate-leaf type), the sinuate-leaf type was confirmed to be distinct in leaf form.

Of the 252 $F_{4:5}$ rows grown in 1992, 173 were classified as normal, 25 segregated three normals: one sinuate-leaf, 15 segregated fifteen normals: one sinuate-leaf, and 39 were considered uniform sinuate-leaf types (Table 1).

The segregation ratios observed in the 1992 $F_{4:5}$ families suggested two recessive genes control the sinuate-leaf trait, with both genes required for expression. However, the fit to a 44:7:1:12 phenotypical ratio was not good with a P-value of P=0.00 (Table 2). When the two segregating classes were combined, the P-value was P=0.15 for a fit of the theoretical 3-class phenotypic ratio of 44 normals: 8 segregating: 12 sinuate (Table 2). We found that combining the normals with the segregating class best fit the theoretical phenotypic ratio of 45 normals: 7 segregating (3:1): 12 sinuate-leaf with a P-value of P=0.31 (Table 2). With the limited number of plants, it is difficult to distinguish nonsegregating normal-rows versus rows that are segregating 15 normals: one sinuate-leaf because of possible contamination.

All F_1 plants of the cross LN92-4353 and T176 had normal leaves, providing evidence that the genetic control of sinuate leaf in LN92-4353 is recessive and not influenced by the cytoplasm. In conclusion, we are convinced that the sinuate-leaf trait is controlled by two recessive genes, with both genes necessary for expression.

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Table 1. Segregation in $F_{4:5}$ families and within families observed in 1992.

	$F_{4:5}$ Plant rows		Individual plants					
			Observed		Expected			
Class	Observed	Expected	normal	sinuate	normal	sinuate	X^2	Prob.[1]
	---------- no. of rows----------							
normal	173	173	-----	-----	-----	-----	-----	-----
3:1	25	28	452	140	444	148	0.58	0.45
15:1	15	4	419	33	424	28	0.85	0.36
sinuate	39	47	-----	-----	-----	-----	-----	-----

[1] Probability of obtaining a higher value of chi-square by chance alone.

Table 2. Progeny in $F_{4:5}$ families tested for goodness of fit to the expected genetic ratios, 1992.

Ratio tested	Observed				Expected				χ^2	Prob.[6]
	normal	3:1[1]	15:1[2]	sinuate	normal	3:1[1]	15:1[2]	sinuate		
44:7:1:12	173	25	15	39	173	28	4	47	32.76	0.00
44:8:12[3]	173	----- 40 -----		39	173	----- 32 -----		47	3.73	0.15
52:12[4]	213	---	---	39	205	---	---	47	1.77	0.18
45:7:12[5]	188	25	---	39	177	28	---	47	2.34	0.31

[1] 3 normal leaf plants to 1sinuate leaf plant.
[2] 5 normal leaf plants to 1sinuate leaf plant.
[3] Segregating classes combined.
[4] Normal and segregating classes grouped.
[5] Normal and 15:1 classes combined.
[6] Probability of obtaining a higher value of chi-square by chance alone.

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Agronomic Evaluation of Lines Derived From a Mutable Pink Flowered Soybean Line

Introduction

Stephens et al. (1993) reported that a pink flower in soybean, controlled by *wp* in the presence of *W1*, was associated with increased protein and seed size. Protein was 4% higher and seed weight was 22% higher for pink flowered lines compared with purple flowered lines. The purpose of this study was to determine the effect of the *wp* gene on agronomic traits and to determine the amount of variation in progeny derived from the mutable line LN89-5320-8.

Materials and Methods

LN89-5320-8 originated as a F_5 derived family from the cross, [Sherman x Asgrow A2943] x Elgin 87. This line was selected because it continued to segregate for flower color, pink and purple. Single plants were selected from within this line based on maturity differences and flower color. This gave rise to four tests: an early and late stable flower color test and a early and late unstable flower color test. In the two stable flower color tests, all lines were considered stable when the flower color remained constant when compared with the original plant. The two unstable tests had lines with flower color that had minor changes, or changed completely when compared with the original selected flower color. Being $F_{5:10}$ and $F_{6:10}$ derived lines, they should be highly homozygous for most genes except for flower color genes.

The early stable flower color test included 30 lines and five cultivars were included as standards: Archer, Bell, Kenwood, Parker, and Sturdy, for a total of 35 entries. The late stable flower color test also had 30 lines and five cultivars were included as standards: Burlison, Jack, Kenwood, LN89-5322-2 (Stephens and Nickell, 1991), and Sturdy, for a total of 35 entries. In the two stable tests, each entry was planted at two locations for two years. Plots were four rows, 4.5 m long, with 76 cm between row spacing and replicated twice. The two center rows were end trimmed to 3 m at the R6 or R7 growth stage (Fehr et al., 1971). All data measurements were recorded from the two center rows.

In the two unstable flower color tests 114 lines were chosen from the progeny of LN89-5320-8-53. Both of these tests had six cultivars that were included as standards: Archer, Burlison, Kenwood, LN89-5322-2, Resnik, and Sturdy. The early test had 35 entries while the late test had 90 entries. Entries in the two unstable tests were planted in two row plots, 4.5 m long with 76 cm between row spacing and replicated twice. The early test was planted at one location due to limited seed while the late test was grown at two locations. These two-row plots were end trimmed to 3 m at the R6 or R7 growth stage. For each plot, approximately 150 viable seeds were planted into each row. Both unstable tests were four row plots and planted at two locations in 1995.

Agronomic traits evaluated were: (i) yield (g $plot^{-1}$ - adjusted to 130 g kg^{-1} moisture), (ii) maturity (date when at least 95% of the pods had mature color), (iii) lodging (scored on the basis of 1=all plants erect to 5=all plants lying flat on soil surface), (iv) plant height at harvest (cm), (v) seed quality, based on the amount of wrinkled and discolored seed (on a scale 1 = excellent to 5 = poor), (vi) seed weight (cg), (vii) seed protein (g kg^{-1}), and (viii) seed oil (g kg^{-1}).

Seed protein and oil were measured on a 25 g seed sample combined from the two replications of each entry. Protein and oil concentrations were determined using infrared reflectance at the National Center for Agricultural Utilization Research, Peoria, Il, and reported on a dry weight basis.

The experimental design was a randomized complete block with two replicates per location combined over two years. In the analysis of variance (ANOVA), the effects of year and location were considered random, while entries were considered fixed. Protein and oil values were analyzed separately, and in these analyses, locations were considered as replications within each year. Statistical computations were carried out using SAS system for Windows (SAS Release 6.08 for personal computers; SAS Institute, Cary, NC). Significance was determined using *F*-test and when necessary the numerator and denominator degrees-of-freedom were calculated as described by McIntosh (1983). Means were separated at the 0.05 probability level using the least significant difference (LSD).

Results and Discussion

The *wp* gene or the pink phenotype was found to be associated with changes in the agronomic traits. In the two stable tests and the late unstable test, seed protein averaged 7 g/kg higher for pink lines than purple lines, 6 g/kg higher than purple/pink lines, and 15 g/kg higher than the standards. The pink lines averaged 3 days later in maturity than the purple lines. Normally, later maturing soybean lines have higher oil concentrations (Miller and Fehr, 1979). The pink lines were significantly later maturing and had higher protein concentration but did not have a significant loss of oil concentration. Seed weight averaged 1.5 cg higher for pink lines than purple lines, 1.6 cg higher than purple/pink lines, and 0.9 cg higher than the standards. Seed yield averaged 56.3 g/plot less for pink lines than purple lines, 108.6 g/plot less than purple/pink lines, and 153.2 g/plot less than the standards.

The results from the early unstable test did not agree with those from the stable tests, this could be due to selection of entries in the early unstable test and genetic makeup of these entries. Selection of lines was based upon highest protein and oil combinations. As a result, twice as many pink lines were selected as purple and purple/pink lines. By selecting twice as many pink lines, the protein and oil percent of the lowest pink lines was not as high as the lowest protein and oil percents of the purple and purple/pink lines. Also the instability of the lines in this test has led to alterations from the original color designations.

In another attempt to evaluate the effect of the *wp* gene, 10 lines were selected based on maturity, 5 pink and 5 purple. From these data, maturity differences were eliminated to create near isogenic subsamples in an effort to look at the genetic effects of *wp*. When *t*-tests were done on the agronomic trait means, two replications for two years at two locations, significance was found in four traits. Seed yield and oil concentration were lower, lodging was decreased, and protein concentration was higher. Seed size was numerically higher but not significant at the 0.05 probability level.

In summary, the *wp* gene is associated with higher seed protein, higher seed weight and lower yield. The pink flowered lines could be used in a breeding program to increase protein and seed size while maintaining seed oil percentage.

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Table 1. Comparison of mean differences in agronomic traits and seed chemical composition by flower color in 1994 and 1995.

	Early stable test			Late stable test		
Trait	Pk† vs P†	Pk vs. P/Pk†	Pk vs. Standards‡	Pk vs P	Pk vs. P/Pk	Pk vs.Standards
Yield (g/ plot)	-64.8*	-127.2*	-245.1*	-149.7	-138.7	-147.2*
Maturity (days)	1.2*	-0.9	-0.2	1.8*	1.0*	4.6*
Height (cm)	-1.0	-3.8*	-12.5*	-5.3*	-4.1*	-5.3*
Seed weight (cg)	1.5*	1.7*	1.3*	2.8*	1.8*	1.0*
Seed quality (score)	0.2	0.1	0.0	0.3*	0.2*	0.2*
Protein (g/kg)	4.0	5.0	16.0*	11.0*	12.0*	14.0*
Oil (g/kg)	-4.0*	-3.0	-5.0*	-3.0*	-2.0	-7.0

* Comparisons significant at $P = 0.05$. (minus indicates pink mean is lower)

† Flower color of entries: Pk = pink, P = purple, P/ Pk = purple/ pink lines

‡ Name cultivars: Archer, Bell, Burlison, Jack, Kenwood, Parker, Sturdy, LN89-5322-2

Table 2. Comparison of mean differences in agronomic traits and seed chemical composition by flower color in 1994 and 1995.

	Early unstable test			Late unstable test		
Trait	Pk† vs P†	Pk vs. P/Pk†	Pk vs. Standards‡	Pk vs P	Pk vs. P/Pk	Pk vs. Standards
Yield (g/ plot)	34.6	-30.4	-259.7*	45.5*	-59.8*	-67.2*
Maturity (days)	0.9	1.9	-1.5	6.1*	1.1*	5.4*
Height (cm)	-2.0	1.3	-10.9*	8.1*	0.0	-0.8
Seed weight (cg)	0.7*	0.5*	1.2*	0.2	1.3*	0.3
Seed quality (score)	0.1	0.0	0.2*	0.1*	0.0	0.2*
Protein (g/kg)	-5.0	-4.0	14.0*	7.0*	3.0*	16.0*
Oil (g/kg)	2.0	1.0	-3.0*	-5.0*	-1.0	-9.0

* Comparisons significant at $P = 0.05$. (minus indicates pink mean is lower)

† Flower color of entries: Pk = pink, P = purple, P/ Pk = purple/ pink lines

‡ Name cultivars: Archer, Burlison, Kenwood, LN89-5322-2, Resnik, and Sturdy

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Genetic Analysis of Brown Stem Rot Resistance in Soybean Germplasm Line LL89-605

Introduction

Monogenic resistance to the disease brown stem rot (BSR) of soybean, caused by *Phialophora gregata* (Allington & Chamberlain) W. Gams, has been identified in soybean accessions from Asia. *Rbs1* was identified in soybean line L78-4094, derived from PI 84946-2 (Sebastian and Nickell, 1985; Hanson et al., 1988). *Rbs2* and *Rbs3* were identified in soybean PI 437833 (Hanson et al., 1988) and PI 437970 (Willmot and Nickell, 1989), respectively. Brown stem rot resistant germplasm line LL89-605 was derived from the cross of PI 437821 and 'Wells II.' (Wilcox et al., 1979) In greenhouse and field studies, BSR resistance of LL89-605 was equal to or better than that of cultivar 'BSR 201' (Tachibana et al., 1983; Nickell et al., 1994). The objectives of this study were to determine the number of loci conditioning BSR resistance in germplasm line LL89-605, and to study the relationship between BSR resistance allele(s) in LL89-605 and *Rbs1*, *Rbs2*, and *Rbs3*.

Materials and Methods

'Century 84' (Walker et al., 1986) was chosen as the susceptible parent as well as the susceptible standard because of its ability to express clearly defined BSR foliar and stem symptoms when inoculated with a type I isolate of *P. gregata* (Gray, 1971). Soybean germplasm line L78-4094 (*Rbs1*), PI 437833 (*Rbs2*) (Hanson et al., 1988) and PI 437970 (*Rbs3*) (Willmot and Nickell, 1989) were used as BSR resistant parents. Progeny in the F2 and F3 generations of the following crosses were evaluated for BSR reaction: cross 1 ['Century 84' (*rbs1, rbs2, rbs3*) x LL89-605]; cross 2 [L78-4094 (*Rbs1*) x LL89-605]; cross 3 [PI 437833 (*Rbs2*) x LL89-605]; and cross 4 [PI 437970 (*Rbs3*) x LL89-605].

Approximately 100 F2 plants and 40 F2:3 families containing 30 plants each were evaluated from each cross. Brown stem rot evaluations of F2 and F3 plants were conducted in the greenhouse between October, 1995 and May, 1996 after inoculation with a type I defoliating isolate of *P. gregata*. The inoculation technique used in this experiment was the *P. gregata* root-dip regime used by Willmot and Nickell, (1989). Inoculated plants were maintained under a 14 h photoperiod ranging from an average nighttime temperature of 18° C to an average daytime temperature of 24 ° C. Each pot received 300 ml of water per day and fertilizer weekly. Plants were evaluated for BSR reaction after 8-9 weeks [R1-R3 growth stage (Fehr et al., 1971)]. Individual pots were rated for incidence of foliar symptoms (% of plants showing foliar symptoms), and individual plants were rated for severity of foliar symptoms on a scale of 1-10 where 1 = no foliar symptoms and 10 = total foliar necrosis. Stem symptoms were measured by splitting each stem lengthwise and recording the height of stem browning, measured in nodes, as a fraction of the total height measured in nodes.

The F2:3 families were classified as resistant, heterozygous, or susceptible based upon results of cluster analysis, as described by Sebastian and Nickell (1985). Families with low foliar symptom means and variances were considered resistant and grouped in a single cluster. Families with intermediate foliar symptom means and high foliar symptom variances were classified as segregating and grouped in a distinct cluster. Families with high foliar symptom means and low foliar symptom variances were grouped in a susceptible class (Sebastian and Nickell, 1985). Gene models for F2 and F2:3 data were tested by chi-square analysis.

Results and Discussion

Resistant standards L78-4094, PI 437833, and PI 437970 developed foliar and stem symptoms, but to a much lesser degree than 'Century 84'. Foliar and stem symptom means of the resistant standards were significantly lower than those of the susceptible standard. Data collected from F2 and F3 progeny from cross 1 supported the presence of a single dominant resistance allele in line LL89-605 (Table 1).

Results from cross 2 indicated that the resistance gene in LL89-605 is non-allelic to *Rbs1* (Table 1). The F2 and F3 generation single plant data from cross 2 showed a poor fit to the expected ratios, but F2:3 family data fit the expected 11:4:1 ratio and supported the model for segregation of two dominant alleles. Poor fit of F2 and F3 generation single plant data may have been the result of

misclassification of resistant plants. (Resistant standard L78-4094 showed susceptible reactions on 23.3 % of the plants). Results from cross 3 were similar to those of cross 2 (Table 1). The ratio of F2:3 families fit the expected 11:4:1 ratio, as indicated by the cluster analysis. However, the F2 and F3 generation single plant data did not fit expected ratios. Again, this may be explained by misclassification of resistant plants. (Resistant standard PI 437833 exhibited foliar symptoms on 10.2% of the plants). The F2:3 family data indicated that the resistance gene in LL89-605 is non-allelic to *Rbs2*.

The F3 plant data and F2:3 family data from cross 4 indicated allelism between the resistance allele in LL89-605 and *Rbs3* (Table 1). Of 40 F2:3 families tested, 38 were classified as resistant or segregating 15:1, and two as segregating 3:1. The F3 single plant data included approximately 8% susceptible plants as compared to 14% expected from segregation of two loci. The F2 plant data from cross 4 fit the expected 15:1 ratio resulting from segregation of two non-allelic genes, and therefore refuted the *Rbs3* model for LL89-605. However, the presence of susceptible plants in the F2 and F3 generations may have been due to misclassification of resistant plants. The *Rbs3* model for LL89-605 was supported by the cluster analysis of cross 4 families.

Misclassification of resistant plants may have resulted from longer exposure to *P. gregata* [8-9 weeks versus 5-6 weeks in previous genetic studies (Hanson et al., 1988; Willmot and Nickell, 1989)]. This length of time may have allowed host resistance mechanisms to be overcome by the fungus. Misclassification may also have resulted from confounding symptoms of powdery mildew in the greenhouse.

BSR resistance conditioned by a single dominant allele is easily transferred into cultivars or germplasm lines used in the production of cultivars. The presence of *Rbs3* in an agronomically acceptable genetic background such as germplasm line LL89-605 (Nickell et al., 1994) may offer another tool to soybean breeders.

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Table 1. Reaction of F_2 and F_3 generation progeny of crosses LL89-605 (BSR resistant) X Century 84 (*rbs1 rbs2 rbs3*), L78-4094 (*Rbs1*), PI 437833 (*Rbs2*), and PI 437970 (*Rbs3*) to inoculation with *Phialophora gregata*.

Generation	Progeny† R	H	S	Ratio tested	Chi-square	*P*
	--------no.--------					
	Century 84 X LL89-605					
F2	84		30	3:1	0.08	0.78
F3‡	742		415	5:3	1.33	0.25
Families						
F2:3 §	10	19	11	1:2:1	0.15	0.93
	L78-4094 X LL89-605					
F2	85		11	15:1	4.45	0.04
F3	935		223	55:9	25.88	0.00
Families						
F2:3	26	13	1	11:4:1	1.88	0.39
	PI 437833 X LL89-605					
F2	95		15	15:1	10.22	0.00
F3	852		262	55:9	82.42	0.00
Families						
F2:3	26	10	4	11:4:1	0.98	0.61
	PI 437970 X LL89-605					
F2	101		8	15:1	0.22	0.50
F3	1092		87	55:9	43.58	0.00
Families						
F2:3	38	2	0	11:4:1	12.91	0.00

† Plants and families were classified as resistant (R), heterozygous (H), or susceptible (S).

‡ Ratio of individual F3 plant BSR reactions.

§ Ratio of F2:3 family BSR reactions.

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RFLP marker assisted identification of *Rps* alleles in F_2 soybean populations

Introduction

Genetic resistance has proven to be one of the most effective methods to combat *Phytophthora* rot in soybean [*Glycine max* (L.)]. Integration of major resistance genes into susceptible elite cultivars has commonly been accomplished through backcrossing. The process of backcrossing in soybean is time consuming and can be questioned as to the amount of progress made. The cultivar into which a desired gene is backcrossed for several generations may not be elite in yield when compared to new cultivars developed in that same time span.

An indirect selection method using molecular markers closely linked to *Rps* alleles has the potential to screen materials on a large scale with acceptable costs (Byrum et al., 1993). In marker assisted selection, inheritance of the resistance trait is followed genotypically rather than phenotypically. RFLP markers tightly linked to specific *Rps* loci have previously been mapped (Diers et al., 1992). The objective of this research was to develop a marker assisted identification technique to detect *Rps* allele segregation in F_2 populations.

Materials and Methods

Near-isogenic lines (isolines) containing *Rps1a,* or *Rps1k* (Bernard et al., 1991) were obtained from the USDA Soybean Germplasm Collection, Urbana, IL. Isolines and elite cultivars Asgrow A3733, Bell, Burlison, Edison, Jack, Resnik, Spry, Thorne, and Yale were grown for leaf tissue in a greenhouse during the winter of 1995 (Table 1). *Rps* isolines and F_2 populations of crosses between polymorphic elite cultivars were grown during the 1995 summer at the Agronomy/Plant Pathology South Farm, Urbana, IL. Approximately 100 plants were individually numbered and tagged in each population. Leaf tissue of each cultivar, *Rps* isoline, and individual F_2 plants was lypholized and stored at -20°C.

Genomic DNA of the parental cultivars and isolines was extracted as minipreps and genomic DNA of F_2 individuals was extracted as micropreps (Dellaporta et al., 1993). Approximately 25 mg of quantified DNA was digested with restriction enzymes *Dra*I, or *Eco*RI. Restricted DNA was electrophoresed in 0.7% agarose gels using 1X TBE (Tris Borate EDTA) buffer. Gels were treated with 0.25 M HCl, denatured with 1.5 M NaCl/0.5 M NaOH, and neutralized with 1 M Tris/1.5 M NaCl, pH 7.4. Gels were blotted to nitrocellulose membrane using capillary action with 10X standard saline citrate (1X SSC is 1.5 M NaCl, 0.15 M sodium citrate, pH 7.0).

Membranes were prehybridized at 68°C in prehybridization solution [6X SSC (0.9 M NaCl, 0.09 M sodium citrate, pH 7.0), 0.5% sodium dodecyl sulfate (SDS), 100 mg/ml denatured calf thymus DNA, 5X Denhardt's solution]. Probe hybridization to the membrane was allowed to occur in the same prehybridization solution overnight at 68°C.

Agar DNA stabs of probes A280 and K418 were obtained from Biogenetic Services (Brookings, SD) and amplified using PCR amplification. Probes were radiolabelled with [α-^{32}P] dATP for approximately 5 hours using the random primer reaction (Feinberg and Vogelstein, 1983). Radiolabelled probes were purified using Biospin column centrifugation (BioRad).

Hybridized blots were rinsed twice for 15 min. at room temperature in low stringency wash (2X SSC, 0.5% SDS, 0.1% sodium pyrophosphate), and in high stringency wash (0.1X SSC, 0.5% SDS, 0.1% sodium pyrophosphate) in a 55°C water shaker for 1-3 hours, depending on intensity of radioactivity measurements. Blots were exposed to X-ray film for 4 to 7 d.

Autoradiographs were scored to determine polymorphisms between elite cultivars that possessed *Rps* genes versus cultivars with *rps*. RFLP bands of *Rps* isolines were used to confirm bands of cultivars possessing known *Rps* alleles. F_2 populations were scored with the same restriction enzyme/probe combinations that displayed polymorphism in the parental cultivars. Each F_2 plant was assigned a genotype according to whether the RFLP bands corresponded to the resistant parent, susceptible parent, or the F_1 heterozygote. RFLP data were subject to chi-square analysis to determine goodness-of-fit to expected genetic models.

Results and Discussion

Several elite cultivars were analyzed using RFLPs to determine if sufficient polymorphism existed between *Rps1k* and *rps1k* genotypes. A blot digested with *Dra*I and probed with A280 revealed a fragment corresponding to *Rps1k* at 3.1 kb in Williams 82 (*Rps1k*). Edison (*Rps1k*), Resnik (*Rps1k*) and Thorne (*Rps1k*) had a band at 3.1 kb. The *rps1k* cultivars, Asgrow A3733, Bell, Jack, Spry, and Yale were lacking the 3.1 kb band.

A blot probed with K418 after digestion with *Eco*RI showed a fragment for Burlison (*Rps11b*) at 4.5 kb which was lacking in Edison and Resnik. Both Edison and Resnik had a fragment at 9.0 kb which was not apparent for Asgrow A3733, Bell, Burlison, Jack, Spry, and Yale.

Ninety individual F_2 plants from the cross Jack x Edison (LNX9112) were scored with enzyme/probe combination *Dra*I/A280 to determine RFLP genotypes that would indicate presence of *Rps1k*. RFLP genotypes were assigned based on bands at 3.1 and 2.6 kb. The 'a' genotype was assigned to Edison and all entries that had a band at 3.1 kb. The F_1 and individuals with bands at 3.1 and 2.6 kb were scored as genotype 'b'. The 'c' genotype was assigned to Jack and all lanes that exhibited a band at 2.6 kb. Of the 64 scorable entries, 16 had the 'a' genotype assigned, 35 had the 'b' genotype designated, and 13 had the 'c' genotype assigned. The chi-square analysis indicated an excellent fit for this data to the expected ratio for single gene segregation (Table 2).

One hundred and ten F_2 plants from the cross Burlison x Edison (LNX8807) were evaluated using *Eco*RI/K418, a combination which found polymorphism existed between *Rps1b* and *Rps1k*. RFLP genotypes were assigned based on banding patterns observed at approximately 9.0 and 4.5 kb. The 'd' genotype was assigned to Edison and all lanes which had a band at 9.0 kb. The 'e' genotype was assigned to the F_1 and all lanes that exhibited a band at 9.0 and 4.5 kb. The 'f' genotype was designated to Burlison and all lanes that had a band at 4.5 kb. Ninety four individuals were scored, of which 20 were genotyped for *Rps1k/Rps1k*, 49 were *Rps1k/Rps1b*, and 25 were *Rps1b/Rps1b*. The chi-square analysis indicates this data conformed well to the expected model (Table 2).

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Table 1. Cultivars and near-isogenic lines containing *Rps* or *rps* alleles.

Cultivars	Alleles	Isoline	Allele	Pedigree†
Burlison†	*Rps1b, Rps3*	L75-6141	*Rps1a*	Williams (6) x SL 11
Edison§	*Rps1k, rps3*	Williams 82	*Rps1k*	Williams (7) x Kingwa
Resnik¶	*Rps1k, rps3*	L76-1988	*Rps2*	Williams (6) x (Harosoy (5) x D54-2437#)
Thorne††	*Rps1k, rps3*	L83-570	*Rps3*	Williams (6) x P.I. 86972-1
Asgrow A3733	*rps1, rps3*	Harosoy 32xx	*Rps3*	Harosoy (5) x P.I. 171442
Bell‡‡	*rps1, rps3*	L85-2352	*Rps4*	Williams (6) x P.I. 86050
Jack§§	*rps1, rps3*	L85-3059	*Rps5*	Williams (6) x P.I. 91160
Spry¶¶	*rps1, rps3*			
Yale##	*rps1, rps3*			

† Numbers in parenthesis refer to number of cycles recurrent parent was crossed
‡ Nickell et al., 1990c
§ McBlain et al., 1991
¶ McBlain et al., 1990
# D54-2437 = CNS, Lincoln, Ogden, Richland, Roanoke
†† McBlain et al., 1993
‡‡ Nickell et al., 1990a
§§ Nickell et al., 1990b
¶¶ Bernard and Nickell, 1992
Nickell et al., 1995

Table 2. Chi-square analysis and correlation of RFLP and pod inoculation data for F_2 populations.

Population	Cross	Genotype Assignment	Proposed RFLP genotype	Observed	Expected	χ^2	P†
LNX9112	Jack x Edison	a	*Rps1k/Rps1k*	16	16		
		b	*Rps1k/rps1k*	35	32		
		c	*rps1k/rps1k*	13	16	0.656	0.844
LNX8807	Burlison x Edison	d	*Rps1k/Rps1k*	20	23.5		
		e	*Rps1k/Rps1b*	49	47		
		f	*Rps1b/Rps1b*	25	23.5	0.704	0.702

† Probability of obtaining a higher value of chi-square by chance alone

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Biosystematics of the genus *Glycine*, 1996.

Table 1 contains a list of the wild perennial *Glycine* species in the subgenus *Glycine* and the annual species in the subgenus *Soja*. In addition, for each species there is a three letter code, 2n chromosome number, cytological standard 1L number, PI number, genome symbol, and distribution. The main points are as follows:

1. Currently, there are 16 wild perennial *Glycine* species. All carry 2n=40 chromosomes except *G. hirticaulis, G. tabacina, and G. tomentella*. These species also have polyploid cytotypes. In addition, *G. tomentella* has 2*n*=38 and 78 cytotypes. The genus *Glycine* is an ancient polyploid having *x*=10. However, the 2*n*=40 plants behave cytologically like diploids. The annual *Glycine* were derived from the perennial forms.

2. The wild perennial *Glycine* species are indigenous to the Australian tectonic plate. Seeds of wild perennial *Glycine* species found outside the plate were brought to these regions by migratory birds via long distance dispersal. Only strong colonizing self breeders such as the polyploid (TAB, TOM) are able to take root in other regions. The diploid like the polyploid seed, most probably, are carried by the birds in their guts, but they simply cannot compete with alien local flora. The annual *Glycine* are indigenous to the Asian plate.

3. The species relationships via genome symbol designations has been clarified. Species having the same letter can be intercrossed resulting in fertile seed. Species having the same letter but different subscripts are taxonomically (morphologically) different species. Usually these species are isolated from one another by paracentric inversions or geography. When hybrids are attempted between species having different letters, the seed must be rescued prior to pod abortion. The resulting seed usually are sterile, lethal or the hybrid plants structurally break down prior to flowering. Based upon our current knowledge, successful hybrids can be made with the soybean using A or a combination of AD or AE genomic plants. Cytogenetic, biochemical, and molecular approaches were utilized in above genomic research activities.

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Table 1. List of the species in the genus *Glycine* Willd., three letter code, 2*n*, accession number, genome symbol, and distribution

		Code	2*n*	IL[1]	PI[1]	Genome[2]	Distribution
				Subgenus *Glycine*			
1.	*G. albicans* Tind. & Craven	ALB	40	889	---------	II	Australia
2.	*G. arenaria* Tind.	ARE	40	689	505204	HH	Australia
3.	*G. argyrea* Tind.	ARG	40	768	505151	A_2A_2	Australia
4.	*G. canescens* F. J. Henn.	CAN	40	434	440932	AA	Australia
5.	*G. clandestina* Wendl.	CLA	40	490	440958	A_1A_1	Australia
6.	*G. curvata* Tind.	CUR	40	791	505166	C_1C_1	Australia
7.	*G. cyrtoloba* Tind.	CYR	40	481	440962	CC	Australia
8.	*G. falcata* Benth.	FLA	*40*	674	505179	FF	Australia
9.	*G. hirticaulis* Tind. & Craven	HIR	40	1246	---------	H_1H_1	Australia
			80	943	---------	------	Australia
10.	*G. lactovirens* Tind. & Craven	LAC	40	1247	---------	I_1I_1	Australia
11.	*G. latifolia* (Benth.) Newell & Hymowitz	LAT	40	373	378709	B_1B_1	Australia
12.	*G. latrobeana* (Meissn.) Benth.	LTR	40	659	483196	A_3A_3	Australia
13.	*G. microphylla* (Benth.) Tind.	MIC	40	449	440956	BB	Australia
14.	*G. pindanica* Tind. & Craven	PIN	40	1251	---------	H_2H_2	Australia
15.	*G. tabacina* (Labill.) Benth.	TAB	40	370	373990	B_2B_2	Australia
			80	----	---------	Complex[3]	Australia, West Central and South Pacific Islands
16.	*G. tomentella* Hayata	TOM	38	398	440998	EE	Australia
		TOM	40	709	505222	DD	Australia, Papua New Guinea
			78	----	---------	Complex[4]	Australia, Papua New Guinea
			80	----	---------	Complex[5]	Australia, Papua New Guinea, Indonesia, Philippines, Taiwan
				Subgenus *Soja* (Moench) F. J. Herm.			
17.	G. *soja* Sieb. & Zucc.	SOJ	40	-----	81762	GG	China, Russia, Taiwan, Japan, Korea (Wild Soybean)
18.	G. *max* (L.) Merr.	MAX	40	cv. Williams 82		GG	Cultigen (Soybean)

[1]IL = A temporary number assigned at Urbana, IL, USA; PI = Plant Introduction number assigned by the USDA

[2]Genomically similar species carry the same letter symbols

[3]Allopolyploids (A and B genome) and segmental allopolyploids (B genome)

[4]Allopolyploids (D and E, A and E, or any other unknown combination)

[5]Allopolyploids (A and D genomes, or any other unknown combination)

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Establishment of a cytogenetic map of soybean: Progress and Prospective

In soybean *[Glycine max* (L.) Merr.], most of the known genes have not been located on chromosomes. Thus, the genetic linkage groups and molecular maps have not been associated with a specific chromosome. Primary trisomics are useful for locating genes on the chromosomes and for associating a linkage group with a particular chromosome in several economically important diploid plant species. The establishment of the first cytological map based on pachytene chromosome analysis (Singh and Hymowitz, 1988) has provided an incentive to isolate and identify primary trisomics ($2n$=41) in this important crop. By pachytene chromosome analysis, 13 primary trisomics of the soybean were identified previously (Singh and Hymowitz, 1991; Ahmad et al., 1992; Ahmad and Hymowitz, 1994). This study reports the isolation and identification of a complete set of primary trisomics ($2n$ = 41) and, tetrasomics ($2n$ = 42) in the soybean. Furthermore, we are trying to develop all primary trisomic lines in a uniform genetic background using the soybean cultivar 'Clark 63' as a recurrent parent.

The primary trisomics were originally derived from the aneuploid lines ($2n$=41-43) produced from different male-sterile lines. Some aneuploid lines were provided by Dr. R. G. Palmer at USDA, ARS, Iowa State University, Ames, Iowa, and others were generated in our laboratory.

The identification of the primary trisomics was based on the association of the extra chromosome in a trivalent configuration, chromosome length, arm ratio, and distribution of euchromatin and heterochromatin at the pachynema stage. The primary trisomics that closely resembled each other in chromosome morphology were verified by meiotic chromosome pairing analysis from plants with $2n$=42 chromosomes. These plants were obtained by crossing two trisomic lines. The two primary trisomics were different when their F_1 plants showed 20II+ 2I, 1III + 19II + 1I, and 2III + 18II at metaphase I in most of the microsporocytes. In contrast, the primary trisomics carried the same extra chromosome when the F_1 plants predominantly exhibited 21II configurations. Since the primary trisomics were of diverse genetic backgrounds, they are being backcrossed into a uniform genetic background by using the soybean cultivar 'Clark 63'.

The tetrasomics were identified from the selfed progenies of the primary trisomics by counting somatic chromosomes. They were confirmed by morphological features and meiotic chromosome pairing.

Currently, we have isolated and identified all possible 20 primary trisomics and related tetrasomics. They are being tentatively designated as Triplo 1 through Triplo 20 and Tetra 1 through Tetra 20 based on chromosome length. Table 1 lists the triplo number, code in University of Illinois at Urbana-Champaign, recurrent generation, and source of identified 20 primary trisomics. Seven primary trisomics including Triplo 7, 9, 11, 15, 17, 18, and 20 were identified for the first time. For the 13 primary trisomics reported previously, an appropriate rearrangement in triplo number was made based on the new cytological evidence. The primary trisomics that originated from $2n$ = 41 chromosome aneuploid lines SRF70:TH216, A88B-13, and SRF70:120 were changed from Triplo 10, 16, and 19 to Triplo 8, 10, and 15, respectively. Table 2. lists the tetra number, code, and source of 20 tetrasomics. Unlike primary trisomics, the tetrasomics expressed various alterations in morphological traits, and the plants from most of the tetrasomics were dwarf and had slow growth, small leaf, and low seed fertility.

These primary trisomics and tetrasomics are being used for locating marker genes to a particular chromosome in our laboratory. This study will eventually associate conventional genetic linkage groups and several molecular maps with the cytogenetic maps as have been done for maize, rice, and tomato. The seeds of the 20 primary trisomics will be released for use when they are in BC_5 generation.

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Table 1. Triplo number, code, recurrent generation with 'Clark 63' and source of primary trisomics (2*n*=41) in soybean.

Triplo	Code	Generation	Source (2*n*)
1	UT95-102	BC_2	Tri C (41)
2	UT95-103	BC_2	KS:TH747 (41)
3	UT95-106	BC_2	KS:TH775 (41)
4	UT95-108	BC_2	Tri D (41)
5	UT95-109	BC_2	Tri A (41)
6	UT95-113	BC_2	KS:TH772 (41)
7	UT96-20	F_1	A84-C1-5-4 (43)
8	UT96-57	BC_2	SRF70:TH216 (41)
9	UT94-20	BC_2	A84-C1-5-8 (42)
10	UT95-142	F_1	A88B-13 (41)
11	UT94-33	BC_2	T170 (41)
12	UT94-86	F_1	T190-47-3 (41)
13	UT95-135	BC_2	Tri S (41)
14	UT94-18	BC_2	A84-C1-5-10 (42)
15	UT94-147	BC_2	SRF70:120 (41)
16	UT95-10	F_1	T190-47-1 (41)
17	UT94-8	BC_2	A84-C1-5-11 (43)
18	UT96-63	F_1	KS:TH745 (41)
19	UT94-39	BC_2	T177 (41)
20	UT93-20	BC_2	A88A-21 (41)

Table 2. Tetrasomics (2*n*=42) derived from progenies of primary trisomics in soybean.

Tetra	Code	Source (2*n*)
1	UT94-103	Tri C (41)
2	UT95-156	KS:TH747 (41)
3	UT94-167	KS:TH775 (41)
4	T92-4-1	Tri D (41)
5	T92-5-9	Tri A (41)
6	UT96-120	KS:TH772 (41)
7	UT96-82	A84-C1-5-4 (43)
8	T92-10-6	SRF70:TH216 (41)
9	UT95-159	A84-C1-5-8 (42)
10	UT93-11	A88B-13 (41)
11	UT94-150	T170 (41)
12	UT96-124	T190-47-3 (41)
13	UT96-131	Tri S (41)
14	Ut95-158	A84-C1-5-10 (42)
15	T92-19-3	SRF70:120 (41)
16	UT96-135	T190-47-1 (41)
17	UT95-165	A84-C1-5-11 (43)
18	UT96-81	KS:TH745 (41)
19	UT95-166	T177 (41)
20	UT96-128	A88A-21 (41)

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Management of the USDA wild perennial *Glycine* collection, 1996

Under a specific cooperative, agreement between the University of Illinois and the USDA, the wild perennial *Glycine* collection is being maintained at the University of Illinois. Table 1 contains the names of the species, somatic chromosome numbers, and number of accessions. The major points are as follows:

1. One shipment containing a total of 75 new accessions was received during 1996. All of these originated in Australia. Seventy-one new accessions were collected this year by Brown, Grace (both from CSIRO/Canberra) and Hymowitz during a trip to central Australia. CSIRO is currently multiplying accessions from this trip; we will receive them at a later date.

2. A number of accessions are difficult to grow through maturity and seed production. To counter this, these lines have been intensively propagated by means of cuttings and grafts onto *G. max* rootstocks. Seeds are surface sterilized and germinated in autoclaved media. This has allowed some accessions of *G. tomentella* to be multiplied for the first time. Some plants are reluctant to set pods - hand pollination of flowers with a toothpick increases pod set in some accessions of *G. canescens* and *G. falcata*.

3. In order to protect employees from dermal exposure to toxins, and in response to the development of multiple pesticide resistance in greenhouse pests, the perennial greenhouse is on a biological control regime. The following measures have been adopted:

a. Screening: all vents have been screened to prevent an influx of nuisance and pest insects.

b. Appropriate chemicals: insecticidal soaps are useful for spot treatments as they have no residual toxicity and no harmful fumes.

c. Predaceous and parasitic organisms:

1. Mealybugs: *Leptomastix* wasps (parasites).
2. Whiteflies: *Encarsia* (parasitic wasp).
3. Thrips: *Orius* predatory bugs.
4. Spider mites: *Mesoselius longipes* (low humidity tolerant) and *Neoselius californicus* (starvation resistant) predaceous mites. The plants are also hosed down to physically disrupt spider mite colonies.
5. Fungus gnats: Gnatrol, a *Bacillus thuringensis* drench.

These organisms may be obtained from any of a number of companies, including the following:

IPM Laboratories, Inc.
P.O. Box 300
Locke, NY 13092-0300

Biotactics
7765 Lakeside Drive
Riverside, CA 92509

Results have been quite encouraging. The high initial costs of the control organisms have been offset by the resistance of the greenhouse to reinfestation and a reduction in chemical expenses. With increasing pesticide regulation and longer reentry restrictions, reducing pesticide levels makes the greenhouse more useful to researchers. As no biological controls exist for fungus diseases, the plants are drenched with Banrot, a broad-spectrum fungicide, and Subdue, a specific for *Phytophthora*, about every six weeks. They are also sprayed with Rubigan to prevent powdery mildew. The beneficial insects seem able to tolerate these fungicides. As required by law, Jean Burridge is a licensed Public Applicator, Demonstration and Research category.

4. During the year, 18 seed requests for wild perennial material were received. A total of 922 packets of seed was shipped. A standard packet contains 5 seeds of an accession. Domestically, seed was shipped to Illinois, Missouri, and New York. Internationally, seed was shipped to Australia, Austria, France, Switzerland, and Taiwan.

5. Voucher specimens of all accessions grown out in the greenhouse were placed in the Crop Evolution Herbarium (CEL).

6. The inventory of the collection is maintained on a Gateway 2000 personal computer.

7. Fifty-seven packets of 50 seed each were sent to the National Seed Storage Laboratory in Fort Collins, Colorado for long term storage. Thus far, 779 accessions have been sent to this facility. Another emergency set, containing 10 seeds per packet, was sent to Dr. R. L. Nelson, Curator, USDA Soybean Germplasm Collection, Urbana, IL. PI numbers are requested when an accession has been successfully multiplied. Seeds of all accessions in the collection are stored in envelopes, in a milk cooler set to 4 degrees Farenheit.

8. To request seed, please write, e-mail, FAX, or call:

a. Dr. T. Hymowitz
Department of Crop Science
University of Illinois
AE-110 Turner Hall
1102 S. Goodwin Ave.
Urbana, Il 61801 USA

b. soyui@uiuc.edu

c. FAX: (217) 333-9817

d. TEL: (217) 333-9454

Table 1. Wild perennial *Glycine* species, somatic chromosome number, and number of accessions.

	Species	2*n*	Number of Accessions
1.	*G. albicans*	40	2*
2.	*G. arenaria*	40	5
3.	*G. argyrea*	40	13
4.	*G. canescens*	40	80
5.	*G. clandestina*	40	138
6.	*G. curvata*	40	9
7.	*G. cyrtoloba*	40	49
8.	*G. falcata*	40	14
9.	*G. hirticaulis*	40	1*
		80	1*
10.	*G. lactovirens*	40	2*
11.	*G. latifolia*	40	46
12.	*G. latrobeana*	40	12*
13.	*G. microphylla*	40	32
14.	*G. pindanica*	40	5
15.	*G. tabacina*	40	14
		80	131
		?	91
16.	*G.tomentella*	38	22
		40	58
		78	55
		80	53
		?	132
			965

* Recalcitrant species with regard to seed multiplication

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Plant exploration trip to the central and region of Australia

From August 15 to September 11, 1996 a USDA sponsored plant exploration trip to the central arid zone of Australia was undertaken to collect wild perennial relatives of the soybean. The team consisted of Dr. Tony Brown and Mr. Jim Grace of CSIRO/Canberra and me. Alice Springs, Northern Territory was our base of operations.

Each plant exploration trip has its own distinguishing features. The main feature of this trip was that we were not permitted to collect *Glycine* species on Aboriginal Land. The Central Aboriginal Land Council proposed to CSIRO that they sign an agreement giving the Land Council forever the intellectual property rights to the collected seed, control over who shall receive any multiplied seed, and control over the nature of the research to be conducted with the seed. CSIRO refused to sign the agreement.

In addition, we were not able to collect in the Uluru-Kata Tjula National Park (Ayers Rock and The Olgas). Although it is a national park, the land is leased for 99 years from the local aboriginal community. Management of the park apparently is a joint local aboriginal community-Government of Australia arrangement.

Lastly, we were informed that prior to collecting on private land in the Northern Territory we would need to obtain permission from the individual owners of each parcel of land. Thus we did not collect on aboriginal land, national parks, or on private property. We did receive a permit to collect on public land under the control of the Northern Territory Parks and Wildlife Commission. Thus, we had to modify about 25% of our travel plans and modify our sampling strategy. The *Glycine* exploration team travelled about 4000 miles.

In spite of all the background noise and collecting impediments, the team collected 71 perennial *Glycine* accessions. Most of the accessions were *G. canescens*. However, we did collect *G. clandestina* (1), *G. falcate* (2) and *G. tomentella* (1). In addition, the team collected 17 accessions of *Gossypium* consisting of three species - *G. australe* (7), *G. bickii, and* G. *sturtianum.* All of the *Glycine* material was sent to Canberra for multiplication. Ultimately, the multiplied seed will be sent to Beltsville for placement in the USDA wild perennial *Glycine* collection maintained at the University of Illinois.

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Coinheritance of Resistance to SCN and SDS in Pyramid x Douglas

Introduction

Sudden Death Syndrome (SDS) is a fungal scorch disease caused by infection of soybean roots by *Fusarium solani* f. sp. *phaseoli*. Leaf chlorosis is often used to determine resistance and susceptibility. Breeding for SDS resistant soybean varieties has been difficult due to the polygenic nature of SDS resistance (Gibson et al., 1994; Chang et al., 1996; 1997). The soybean cyst nematode (SCN) disease is caused by *Heterodera glycines* Ichinoe. Resistance is usually determined by counting the number of cysts on roots. Foliar symptoms of seedlings vary from slight stunting to severe chlorosis and death.

Varietal resistance is the most effective control method for both SDS and SCN. Varieties resistant to SCN race 3 are often resistant to SDS (Gibson et al., 1994). Coinheritance of resistance to SDS and SCN has been reported in Essex x Forrest (ExF) (Chang et al., 1997) and Pyramid x Douglas (Njiti et al., 1996). The soybean cultivars Forrest and Pyramid are both SDS and SCN resistant whereas Essex and Douglas are SDS and SCN susceptible.

The SDS project aims to improve resistance to SDS in northern soybean germplasm through marker-assisted breeding. Marker-assisted breeding uses DNA markers that are closely linked to the resistant genes to predict phenotype. DNA markers offer advantages with respect to increased numbers of loci detectable and mapped, overall phenotypic neutrality, and the ability to score the plant at any developmental stage.

In selecting for SDS resistance, genes conferring resistance to SCN can be beneficial, due to genetic linkage between loci underlying resistance to SDS and SCN (Chang et al., 1997). However, both cultivar trials and inheritance studies show that the beneficial effect of resistance to SCN on resistance to SDS is often environmentally sensitive (Njiti et al., 1996). Therefore, not all alleles or all loci conferring resistance to SCN are effective in conferring resistance to SDS. The objective of this study was to map QTL underlying resistance to SCN and SDS in Pyramid x Douglas and compare with the effect of the alleles and loci already discovered in Essex x Forrest.

Materials and Methods

Plant materials include Pyramid by Douglas (PxD) F_6 derived population of 90 recombinant inbred lines. Pyramid is SDS and SCN resistant and Douglas is SDS and SCN susceptible. Trait data were the same as reported previously (Njiti et al., 1996). The molecular marker techniques used in the report include, Simple Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphisms (RFLP) (see Chang et al., 1997). The linkage data obtained was analyzed by Map-Maker version 3.0. Intervals governing SDS response were detected using one way ANOVA and Map-maker-QTL version 1.1.

Results

This report summarizes the data from analysis of PxD with a total of 51 loci compared to ExF analyzed with 131 markers (Chang et al., 1997). Four PxD loci were identified by RFLP markers, 10 by microsatellite markers and 37 loci by RAPD bands. In total, 14 discrete loci were mapped to four linkage groups. The mean interval was about 30cM.

Of the 90 RILs characterized, 43 were resistant to SCN race 3, 47 were susceptible to SCN (of which three segregated for SCN resistance). Of the 43 lines resistant to SCN race 3, 29 were resistant to SCN race 14. Resistance to SCN race 3 of about 50% of RILs could indicate that Pyramid contains SCN resistant genes *rhg5* (from PI88788) (Rao Arrelli et al., 1991) in addition to *rhg1* and *Rhg4* (from Peking) (Webb et al., 1995; Chang et al., 1997) if one gene was redundant. Marker data suggests this is the case (Table 1). RAPD marker $OA12_{1000}$ and linked (20 cM) RFLP marker A085H identified the QTL corresponding to *Rhg4* on linkage group A2 accounted for 17% and 9% of total variation in SCN race 3 score. Markers $OD04_{950}$ and linked (39 cM) SATT38 allowed the SCN resistance QTL corresponding to *rhg1* to be identified on linkage group G and accounted for 17% and 9% of the variation. The RAPD marker, $OG01_{390}$ was significantly associated with SCN race 3 resistance accounting for 14% of the variability. Linkage (31 cM) of RAPD marker $OG01_{390}$ with the microsatellite

marker SATT71 allowed the QTL to be assigned to linkage group D in PxD mapping population. This QTL may correspond to *rhg5* since it has not been described in lines deriving SCN resistance from Peking and allelic sources of SCN resistance (Webb et al., 1995; Concibido et al., 1996; Chang et al., 1997). All three QTL were identified as major QTL for resistance to SCN race 3 and race 14. The three genes in Pyramid are mutually redundant. To be resistant (IP<10%) a RIL need only possess two of the three loci (A, G, D). Lines with all three are not more resistant than those with two. Therefore, the gene pyramid in Pyramid does not increase resistance in our assay but does increase heritability of resistance.

One major SDS QTL has been assigned to the *rhg1* region on linkage group G in ExF. However, in PxD the locus near SATT38 on linkage group G explains resistance to SCN (races 3 and 14) but not SDS resistance. The locus near $OG01_{390}$ on linkage group D explains resistance to SCN race 3 and 14 but not SDS resistance. Therefore, only the weak association between SDS and SCN resistance on linkage group A2 can explain the association between resistance to SCN in PxD and varietal and inheritance studies where SCN resistance is derived from PI88788. Clearly, SDS and SCN resistance gene clustering is over a larger genetic distance in PxD (about 20 cM) than in ExF (2 cM). To date no strong QTL conditioning resistance to SDS have been found in PxD that are independent of SCN resistance, although 9 environment specific loci have been detected. In addition, because $OD04_{950}$ maps to linkage group G in the approximate position of the second SDS resistance QTL in ExF (Chang et al., 1996) the two major QTL for resistance to SDS in ExF do not appear to condition resistance to SDS in PxD. Therefore, inheritance of resistance to SDS is quite different in PxD and ExF. Saturation of the PxD and ExF maps with AFLP and SSR markers will verify these preliminary conclusions and identify the SDS resistance QTL and race 14 specific SCN resistance QTL.

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Development and utility of sequence characterized amplified regions (SCARS) from RAPD markers linked to SDS and SCN QTLs

Introduction

Due to the losses in seed yield caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans., type A (SDS) and the nematode *Heterodera glycines* (SCN) (Wrather et al., 1995, 1996), the identification, selection, and isolation of resistance genes are being pursued. Marker-assisted selection and gene isolation will provide means for gene introgression into elite soybean varieties.

Resistance to SDS is controlled by several genes, is incomplete and quantitative (Chang et al., 1996, 1997; Njiti et al., 1996). With the objective of identifying and localizing QTL associated with SDS resistance, 131 polymorphic markers were scored for association with yield and SDS resistance in Essex x Forrest. Four QTL accounted for approximately 65% of the total phenotypic variability in mean disease index (DI), 50% in mean disease severity (DS) and 35% in mean yield. Two of these QTL were identified by RAPD markers $OIO3_{450}$ and $OG13_{490}$ (molecular linkage group 1G) and by $OEO4_{450}$ and $OEO2_{1000}$ (molecular linkage group 2G) (Chang et al., 1996).

Resistance to SCN is oligogenic and complex. Genetic mapping has identified three resistance QTL for SCN on linkage groups G, M, and A (Concibido et al., 1996; Webb et al., 1995; Chang et al., 1997). Coinheritance of field resistance to SDS and SCN has been demonstrated in a variety of studies (Njiti et al., 1996 and references therein). Chang et al., (1997) showed that the marker $OIO3_{450}$ identified a QTL for SCN resistance (R^2 = 14%) in a region on linkage group G that was strongly associated with SDS disease index (R^2=20%) partly accounting for the coinheritance of the two resistance traits. Doubler et al., (1997, this issue) have excluded pleiotropy and shown that this region contains a cluster of resistance genes.

Due to the proximity of the marker $OIO3_{450}$ to the clustered resistance genes for SDS and SCN on linkage group G, our objective was to convert this marker into a SCAR for use in marker assisted breeding for resistance to SDS and SCN. Since the marker would be used to score thousands of individuals, SCAR markers or their derivatives are most effective when used in a high throughput DNA assay. The utility of SCAR markers in soybean and the potential use of a relatively new high throughput assay are discussed.

Materials and Methods

Amplification conditions for RAPD analysis and DNA isolation are described in Hnetkovsky et al (1996). RAPD marker $OIO3_{450}$ was excised from a 1.4% agarose gel and purified using the GENE CLEAN™ kit from Promega according to the manufacturer instructions with one modification. A final rinse of the pellet with 70% ethanol was included. This step was necessary as it removed interfering salts for subsequent amplification and ligation from the WASH™ solution.

Amplification conditions for the SCAR primers are as follows: Twenty five µl reaction volume contained 10 mM Tris-HCL, pH8.3, 50mM KCl, 200µM dNTP, 0.15µM each primer, 1 unit AmpliTaq™ (Perkin Elmer), 2.4 mM Mg^{2+} and 20-40 ng template DNA. The samples were amplified according to 1 cycle of 95°C, 3 minutes; 35 cycles of 95°C, 30 seconds, 50°C, 30 seconds, 72° 2 minutes; 1 cycle of 72°C, 5 minutes.

Results and Discussion

RAPD profiles obtained from Essex and Forrest with the primer OIO3 showed approximately 12 bands of varying intensity ranging from 200 to 1000 bp (Fig. 1, lanes 3, 4). Closely spaced bands are typical of RAPD profiles and extracting a clean fragment for elution presents some difficulty. In case of $OIO3_{450}$, excision and re-amplification of the polymorphic band from Essex produced two closely spaced bands (Fig. 1 lanes 1, 2). These extra bands arise either from amplification artifacts that are extremely reproducible, or from close, contaminating bands that are efficiently amplified, or from internal primer sites in the RAPD product. However, sequence analysis (see below) showed no internal sequence sites. Furthermore, contaminating bands have also been observed when

the products were separated on a polyacrylamide DAF gel (Prabhu, 1995) suggesting that these extra bands do not necessarily arise from closely spaced bands. Furthermore, a Southern blot (Fig. 3) with labeled purified fragment (see below) revealed a pattern of low complexity. To remove the contaminating band, the re-amplified $OIO3_{450}$ fragment was separated on a 2% agarose gel, and only a small interior portion of the band of interest was excised. Upon re-amplification a single band was obtained that corresponded in size to the original polymorphic band (Fig. 1 lanes 5, 6).

Several unsuccessful attempts at cloning the purified fragment using a T-tailed vector, confirmed previous findings of Prabhu (1995) that all arbitrary primer amplification products do not have an extra A tagged on by the Taq polymerase. The nucleotide added at the 3' end depends on the type of nucleotide present at the 5' end of the primer, a G, C, or A ("C" in case of OIO3) can produce fragments with an extra A, C, G, or T. The purified OIO3 was successfully cloned using blunt ends according to the procedure described in Prabhu (1995). Sequencing of the cloned fragment showed that the Essex allele was 512 bp, therefore OIO3 should me renamed $OIO3_{512}$.

Based on the sequence of the Essex allele, a set of SCAR primers that included the original RAPD primer sequence were used to amplify Essex and Forrest genomic DNA. A non-polymorphic band of identical molecular size (512 bp) was amplified from both parents (Fig. 2). Restriction digests of the fragment with a variety of enzymes showed no polymorphisms (data not shown). Alleles from both Essex and Forrest amplified by the SCAR primers were amplified, excised, eluted, and sequenced. Sequence analysis showed that the two alleles were identical, 61% AT in composition interspersed with stretches of polyA and polyT. Amplification of the progenitors of Essex (E) and Forrest (F) CNS (C), Lee (L), S100 (S), varieties that are common to both Essex and Forrest, Bragg (B), Jackson (J), Dyer (D) showed no allelic variation in terms of molecular size (Fig. 2). Furthermore Peking, Pyramid and Douglas also exhibited identical size bands. Sequence analysis of the alleles from the progenitors is ongoing.

These results confirm the lack of genetic diversity found by several researchers and breeders in cultivated soybean (Keim et al., 1989). Recent findings of strong sequence conservation in microsatellite flanking regions in soybean (P. Cregan, personal communication) suggests that the level of sequence divergence in elite varieties is very low. It seems likely therefore that technology utilizing SCAR primers designed on the basis of single nucleotide differences will have to be exploited.

Ongoing efforts to obtain $OIO3_{512}$ SCAR primers involve use of "anchored PCR" to identify the polymorphic nucleotide between Essex and Forrest and search for sequence differences in flanking regions of the OIO3 sequence. To increase throughput in our marker-assisted selection, we will utilize the "TaqMan"™ assay (PE Applied Biosystems) and/or SNPs (GeneScreen, MD), both fluorescence assay systems are based on the 5' nuclease activity of Taq DNA polymerase. Briefly, the TaqMan method utilizes a oligonucleotide primer that is attached to a fluorescent reporter and a quencher molecule. If the allele corresponding to the primer is present, the polymerase displaces the dye molecule during the extension phase of the amplification resulting in an increase in fluorescence. A single nucleotide difference in the other allele will not result in the displacement of the dye and will remain quenched. Both detection systems provides real time quantitative results and allows detection of heterozygote genotypes.

Conclusions

Based on the sequence analysis of the OIO3 alleles from Essex and Forrest, sequence differences in soybean could be as low as 1 in 500 bases. Marker assisted selection using SCAR primers based on single nucleotide differences is currently being explored.

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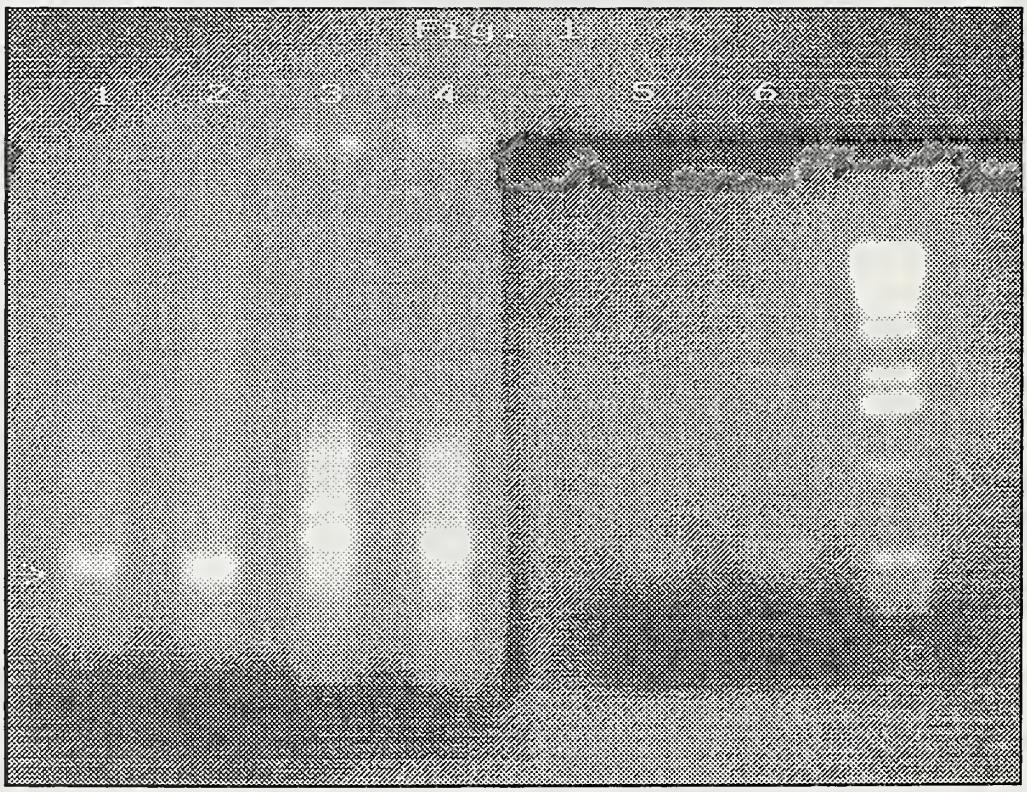

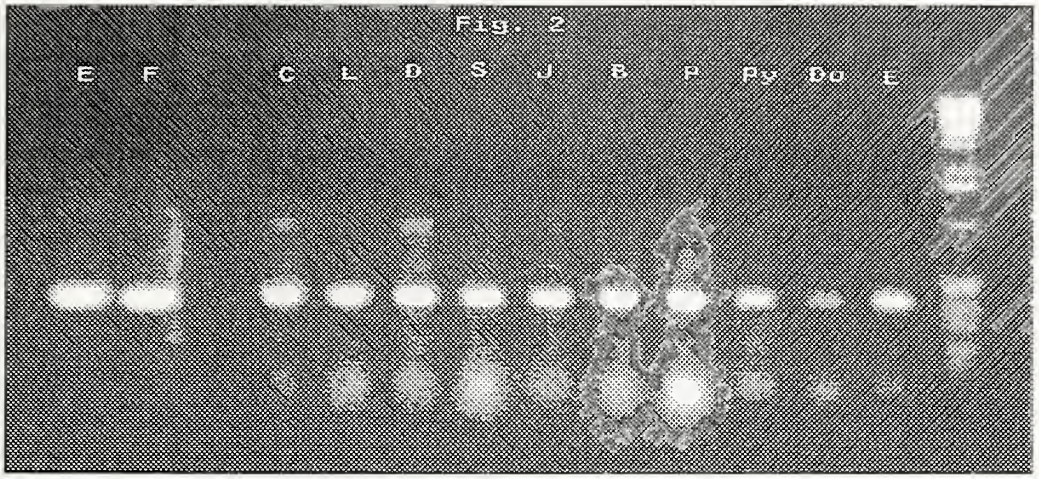
Fig. 2
E F C L D S J B P Py Do E

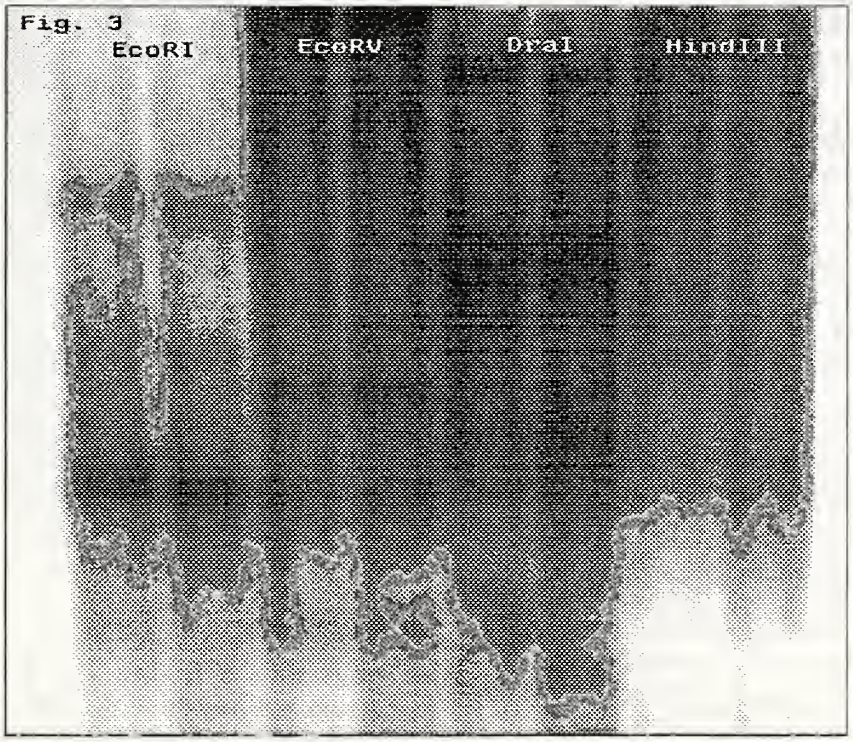
Fig. 3
EcoRI
EcoRV
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An Effective Greenhouse Assay for Field Resistance to SDS.

Introduction

Soybean sudden death syndrome (SDS) caused by *Fusarium solani*, f.sp. *phaseoli* (O'Donnell and Gray, 1995) infects and produces leaf symptoms in all field tested soybean genotypes (Njiti et al., 1997, Gibson et al, 1994). However, differences exist in the frequency and severity of leaf and root symptoms among cultivars (Gibson et al., 1994). Field resistance to infection is rate-reducing and is conditioned by a few major QTL (Njiti et al., 1997; Chang et al., 1996).

Chemical and cultural controls for SDS have not been successful (Wrather et al., 1995). The use of resistant cultivars is the most effective method of control (Gibson et al., 1994). Resistance in the field is polygenic (Njiti et al., 1996; Chang et al., 1996). However, Stephens et al. (1993) inferred that resistance in 'Ripley' (Cooper et al., 1990) was conditioned by a single dominant gene, *rfs*.

Breeding for field resistance to SDS is a major task facing plant breeders in mid-western USA. Selection is complicated by the continuous nature of the trait and its interactions with the environment (Njiti et al., 1996). Field selection is inefficient, time-consuming, and expensive. Marker-assisted selection (MAS), complemented by field testing is more efficient but time-consuming. MAS, complemented by greenhouse testing would be ideal. However, current greenhouse assays do not predict field performance (Torto et al., 1996).

There are two commonly used greenhouse assays; the *F. solani* infested oat seed assay (Lim, 1991) that does not produce consistent symptoms; and the *F. solani* infested sand/cornmeal (Killebrew et al., 1988) mixture that produces consistent but severe symptoms in all genotypes but does not predict field performance (Torto et al., 1996). A consistent greenhouse SDS assay that can predict field performance would complement MAS in developing cultivars with strong SDS resistance. Therefore, our goal was to improve the relationship between field and greenhouse SDS disease severity using a range of inoculum concentrations in the sand/cornmeal greenhouse assay for resistance to *F. solani* applied to soybean RILs.

Materials and Methods

The genetic material used included 30 F_5 derived lines (a subset of a population of 100 lines) from the cross of 'Essex' (Smith and Camper, 1973) x 'Forrest' (Hartwig and Epps, 1973). Three groups of 10 RILs were selected based on 5 locations DS means: field SDS resistant (10 best), field SDS moderate (10 middle) and field SDS susceptible (10 worst) were established based on 5 location means.

A 1:1 mixture of cornmeal and silicon dioxide was infested with *F. solani* (ST90) and allowed to incubate for 10 days after O'Donnell and Gray (1995). The mixture was used to infest a sterile 1:1 mixture of sand/soil at three different inoculum:sand/soil (v/v) ratios to establish inoculum concentrations of: high (1:40, about 10^4 hyphae per cm^3, recommended concentration), medium (1:80, about $5x10^3$ hyphae per cm^3), and low (1:120, about $3.3x10^3$ hyphae per cm^3). Two-week-old seedlings were transplanted into the inoculated mixture in styrofoam cups and kept saturated for four weeks. The experiments consisted of two runs in a randomized complete block with 2 replications. Experiments were rated for SDS disease severity (DS) at 21 and 28 days after transplanting. The data taken at 21 days gave maximum differentiation among genotypes and was used for data analysis. Data analysis included analysis of variance, mean comparison, correlations, and comparison of greenhouse DS with SDS field resistance (Chang et al., 1996) by one-way ANOVA.

Results

The mean DS (Tables 1 and 2) indicate that the disease was severe in the high, and to some extent the medium concentration, making it difficult to differentiate resistant from susceptible genotypes. There was a significant difference in DS means among genotypes for inoculum concentration and run (Tables 1 and 2). The DS means of all inoculum concentrations were significantly different from each other. The DS means for both runs in the low and medium inoculum concentration were significantly different. The high inoculum concentration DS means could not be compared as there was lost data

in run 1 (Table 2). Run to run difference implies that there is a run to run variability in the assay that may be caused by the actual amount of the pathogen used, its pathogenicity or micro environmental factors that influence infection or disease expression. However, the genotypes are affected proportionately by these factors as there was no significant genotype by run interaction (Table 1). There was a concentration by genotype interaction (Table 2) implying that genotypes were responding differently as the amount of pathogen was varied.

By lowering the amount of pathogen in the soil, the amount of variability among genotypes was greatly increased, with the low inoculum concentration showing significant genotype variation in all runs (Table 1). This increased variation resulted in higher heritability estimates and hence better chance of successful selection in the greenhouse. The correlation between field and greenhouse DS also became better as the inoculum concentration was lower, with the low inoculum concentration showing a significant correlation in both runs while the medium inoculum concentration showed a significant correlation in run 1 only (Table 3). The ability to predict field performance in the greenhouse was improved, as 8 of the 10 field SDS resistant lines were also greenhouse SDS resistant and 9 of the 10 field SDS susceptible lines were also greenhouse SDS susceptible (Table 4). Selection among the field SDS moderates was more difficult as 4 of the 10 lines were misclassified in the greenhouse as SDS resistant and 2 were misclassified in the greenhouse as SDS susceptible. DS from the low inoculum concentration was associated with most of the DNA makers that identify QTL conditioning SDS resistance in the field (data not shown) (Chang et al.,1996). Therefore, we conclude that low inoculum concentration greenhouse screening of SDS can be effectively used as a complementary technique for MAS in some progeny populations.

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Table 1. Analysis of variance.

Within run and inoculum concentrations

		Run1			Run2		
Source	df	Low	Medium	High	Low	Medium	High
Replication	1	1.9	1.3	-	19.4***	9.1*	7.5*
Genotype	29	3.2*	2.0	-	3.1***	3.4	2.7
Error	29	1.2	1.2	-	1.3	2.0	1.8
	Line mean Heritability						
		62%	40%	-	58%	42%	34%

Across run within inoculum concentration

Source	df	Low	Medium	High
Run	1	6.0**	24.3***	-
Rep (Run)	2	10.7**	5.2	-
Genotype	29	4.6***	2.3	-
Run* Genotype	29	1.5	2.4	-
Error	26	1.7	2.3	-

Across inoculum concentrations within run

Source	df	Run1	Run2
Replication	1	0.1	4.9
Concentration	2	82.5***	345.0***
Genotype	29	2.4*	4.3***
Conc. *Rep	2	1.8	11.1***
Conc. *Genotype	58	2.4*	2.3*
Error	43	1.4	1.3

*,**,*** = Significant at Alpha=0.05, 0.01, and 0.001 respectively.
- = Missing values

Table 2. Disease severity mean comparisons across runs and inoculum concentrations and comparison of SDS genotype classes.

	Run1	Run2	
Low	2.9 A† b‡	2.2 B† a‡	
Medium	5.1 B† b‡	4.2 A† a‡	
High		-	7.3 C†

Comparison of genotype classes

	Run1§	Run2§	Average§
Field resistant	2.2 A	1.5 A	1.9 A
Field moderate	2.6 A	1.5 A	2.0 A
Field susceptible	3.7 B	3.2 B	3.5 B

† Means within a column with same letter are not different.
‡ Means within a row with the same letter are not different.
§ Means within a column with the same letter are not different

Table 3. Correlation between field and greenhouse disease severity (DS).

Concentration	R	R^2	Probability	Means
Run 1				
Low	0.47	0.22	0.0090	2.9
Medium	0.53	0.28	0.0020	5.1
High	-	-	-	-
Run 2				
Low	0.76	0.58	0.0001	2.2
Medium	0.19	0.04	0.3100	4.2
High	0.04	0.00	0.8410	7.3

- = missing values.

Table 4. Frequency of correspondence between SDS field resistant, moderate and susceptible and SDS greenhouse resistant, moderate and susceptible in the low inoculum concentration based on run means.

	Field			
Greenhouse	Resistant	Moderate	Susc.	Greenhouse DS Means
Resistant	8	4	1	1.6
Moderate	0	4	0	2.2
Susceptible	2	2	9	3.6
Field DS Means	1.2	1.4	2.1	

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Mapping Loci Underlying Yield in Illinois.

Introduction

In crop species such as corn, barley, and tomato, where molecular mapping is more advanced, major yield QTL have been detected within elite germplasm of high yield potential (Stuber et al., 1992; Ajmone-Marsan et al., 1995; Backes et al., 1995; Goldman et al., 1995). The individual yield QTL can explain up to 25% of yield variability and may be stable in different genetic backgrounds and environments. Similar QTL are likely to exist within adapted soybean cultivars. However, molecular mapping of yield QTL in soybean can be confounded by segregation of QTL for maturity, growth habit or morphology (major yield determinates) and their interacting factors. Furthermore, mapping yield in germplasm of low yield potential can be misleading.

Molecular markers linked to loci which increase yield have been detected in soybean in Minnesota, Chile (Mansur et al., 1996), Iowa (Keim et al., 1990), and Illinois (Hnetkovsky et al., 1996). The usefulness of the QTL detected in Minnesota, Chile and Iowa to Illinois is questionable because the parents were not of high yield potential, segregated for maturity and growth habit, and are not adapted to southern Illinois (MG 4-5). The QTL detected in Illinois were under SDS disease pressure and resulted entirely from the effect of SDS resistance loci in *Fusarium solani* infested fields. These loci may not increase yield in the absence of the SDS pathogen. Our goal was to identify loci, independent of SDS resistant loci that are capable of increasing soybean yield in Illinois using the Essex x Forrest (ExF) RIL population that yields highly and segregates for yield but not for maturity, growth habit or morphology.

Materials and Methods

The genetic material used include 94 F_5 RILs derived from the cross of 'Essex' (Smith and Camper, 1973) x 'Forrest' (Hartwig and Epps, 1973). The experiments were planted in a randomized complete block design in 4 row plots, 3 replications and 3 locations with no history of soybean sudden death syndrome (SDS) leaf symptoms and low SCN counts. Rows were end-trimmed to 6.1 m and the middle two rows were harvested with a 2-row combine. Yield (Mg ha^{-1}) was calculated for each plot. The mean yield for each RIL was calculated. The 131 DNA markers (Hnetkovsky et al., 1996; Chang et al., 1996, 1997) were compared with yield data using one-way ANOVA.

Results and Discussion

There was a significant difference in yield among the three locations with location mean ranging from 2.75 Mg ha^{-1} in Carbondale to 4.42 Mg ha^{-1} in Ridgway (Table 1). There was no genotype by location interaction. This means that environmental factors that were influencing yield in any location had similar effect on all genotypes. There was no significant variability in yield among RILs in Carbondale, the low yield location. Drought stress affected the genotypes with high yield potential more than those with lower yield potential. Essex and Forrest were almost equal in yield in every environment but Carbondale. There were transgressive segregants in every environment and the mean although the population was skewed toward lower yield than either parent.

Markers associated with location-specific yield were found on 10 linkage groups (data not shown). Markers associated with the mean yield were found on five linkage groups (LG) (Table 3); K636 (LG.A), $OP03_{1330}$ (LG.SIU11), $OC19_{400}$ (LG. unknown), $DAF8.6c_{350}$ (LG. SIU6) and BNG122 (LG. G). These markers may be linked to QTL underlying high yield potential. The QTL can individually explain from 5-25% of the variation in yield (0.06-0.28 Mg/ha). Jointly, they could explain about 65% (0.72 Mg/ha) of the mean yield variability.

Summary

Most of the associations detected were weak and environment specific. However, very few markers were associated with yield in Carbondale where yield variation was not significant (Table 1, Table 2) suggesting positive associations in the other environments are not errors. Low yield variation may have been related to drought stress in this location in 1996.

At three of the five markers that were associated with mean yield, Forrest provided the beneficial allele. Essex provided the beneficial allele at two loci. The highest yielding RILs derived QTL from both parents. Only Bng122 had previously been associated with yield in *F. solani* and SCN infested locations (Chang et al., 1996; 1997). The yield benefit in this study may result from the SDS or SCN

resistance, but no disease symptoms were visible. The map locations of the five major QTL suggests they are not conditioning maturity (Hnetkovsky et al., 1996; Chang et al., 1996; Mansur et al., 1996) and are different from the yield QTL in those studies. It is noteworthy that the RIL mean yield was 3.58 Mg/ha, much higher than the 1.95 Mg/ha reported for Minsoy x Noir. The ExF cross is between related parents and in this is typical of the majority of breeders crosses. Both cultivars are not modern, released in 1973, but the same QTL may segregate in modern cultivars and progeny populations derived from Hartwig (Forrest[3] x PI437.654) and Renick or Flyer (Williams x Essex) among others. In order to confirm that these loci contribute to high yield in adapted soybean cultivars, a larger RIL population or small NIL population may be needed. Several such populations have been developed at SIUC for testing in 1997.

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Table 1. Combined location mean yield (Mg/ha) for Essex, Forrest, and their derived RIL population.

Location	Means† Range		RIL Means† Mean†	Essex mean†	Forrest
Ridgway	4.4	A	3.6 - 4.9	4.5	4.4
Desoto	3.6	B	2.8 - 4.3	3.9	3.7
Carbondale	2.8	C	1.6 - 3.8	2.9	3.7
Average	3.6		3.1 - 4.2	3.8	3.9

† Numbers in columns followed by letters are significantly different.

Table 2. Mean square (MS) values from analysis of variance within and across locations.

Source	df	Ridgway	Desoto	Carbondale
Within location				
Rep	2	0.002ns	1.69***	9.27***
Genotype	93	0.201*	0.258***	0.302ns
Error	186	0.150	0.258	0.302
Combined location				
Location	2	151***		
Rep (location)	2	2.78***		
Genotype	93	0.35***		
Genotype* location	186	0.209		
Pooled error	558	0.237		

*, **, ***, Significant at P = 0.05, 0.01 and 0.001 respectively.

Table 3. Markers that are associated with mean yield across three locations in the Essex x Forrest RIL population.

	BNG122	K636	$OP03_{1330}$	$OC19_{400}$	$DAF8.6c_{350}$
Linkage Group	G	A	SIU11	Unknown	SIU6
Ridgway					
Prob.	ns	0.05	ns	0.0001	0.03
R^2	-	0.15	-	0.26	0.07
Essex†	-	4.6±0.1	-	4.1±0.2	4.4±0.3
Forrest†	-	4.3±0.3	-	4.5±0.2	4.6±0.2
Desoto					
Prob.	ns	ns	0.01	ns	ns
R^2	-	-	0.07	-	-
Essex†	-	-	3.7±0.3	-	-
Forrest†	-	-	3.5±0.4	-	-
Carbondale					
Prob.	ns	0.04	ns	ns	ns
R^2	-	0.16	-	-	-
Essex†	-	3.1±0.3	-	-	-
Forrest†	-	2.6±0.4	-	-	-
Means					
Prob.	0.03	0.009	0.04	0.002	0.05
R^2	0.11	0.24	0.05	0.17	0.06
Essex†	3.7±0.2	3.9±0.2	3.8±0.2	3.5±0.2	3.6±0.2
Forrest†	3.8±0.2	3.6±0.2	3.6±0.3	3.8±0.2	3.8±0.2

† Allelic means for the Essex and Forrest allels are in Mg ha^{-1}.

ns = No significant association at P ≤ 0.05.

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Qualitative Inheritance of Quantitative Trait Loci.

The continuous variation in phenotype observed for most important traits in agriculture is caused by the segregation of independent polygenes of small effect (Patterson et al., 1990). Polygenes can be detected and mapped within 10-20 cM intervals as quantitative trait loci, particularly with DNA markers. Such localization is sufficient for some aspects of their study and manipulation. However, highly accurate estimates of the QTL map location, within less than 1-2 cM is necessary for effective marker assisted selection, distinguishing gene clustering from pleiotrophy and the physical mapping of genes underlying QTL.

Fine mapping of QTL cannot be achieved in F_2 or RIL populations (Darvasi et al., 1993; 1995). Even for QTL of large effect scored in populations containing more than 1,000 recombinants and with marker saturation to less than 1 cM the QTL cannot be localized to intervals smaller than 10 cM. Fine mapping relies on the analysis of derived sub-populations in which the QTL can be further localized. Such methods include; substitution mapping in BC_2F_1 lines (Patterson et al., 1990); mapping in near isogenic lines (Bentolila et al., 1991); mapping in advanced intercross lines (Darvasi et al., 1995); mapping in recombinant backcross inbred lines (Eshed and Zamir, 1995); and mapping in recombinant inbred sub-line populations (Haley et al., 1994). Each of these related methods involves the isolation of the QTL to a 30-40 cM region using molecular markers followed by analysis of recombination within this region in an otherwise homogeneous genetic background. A single cycle of recombination analysis can place QTL within intervals less than 1 cM. Subsequent cycles generated by intercrossing novel recombinants will reduce the interval size still further, given sufficient marker density and larger population sizes.

A single chromosomal region in Forrest underlies coinheritance of field resistance of soybean (*Glycine max* (L.) Merr.) to sudden death syndrome (SDS) (caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans.) and soybean cyst nematode (SCN) race 3 (caused by *Heterodera glycines* Ichinohe). Our objective was to accurately map the loci underlying this coinheritance and distinguish gene clustering from pleiotrophy. We show here the isolation and separation of two clustered QTL (and the underlying loci) in isogeneic lines derived from residual heterogenity within a single RIL.

Materials and Methods

The cross of 'Essex' (Smith and Camper, 1973) by 'Forrest' (Hartwig and Epps, 1973) (ExF) was made and an F_5 derived population of 100 RILs generated. During the studies described herein the RILs were advanced to the $F_{5:13}$ generation from never less than 300 plants per generation. Residual heterogeneity within RILs was about 8% as detected by codominant markers at the $F_{5:9}$ (Hnetkovsky et al., 1996; Chang et al., 1996). For the RFLP marker Bng122D six RILs that were heterogeneic were detected. In this report one of these RILs ExF34 was used to extract subline populations of 40 individuals at the $F_{5:9}$ generation by seed to row descent. Subline populations from the $F_{5:9:11}$ to $F_{5:9:13}$ were used to test for resistance to SDS and SCN. SDS disease index (DX), was determined in three replicated locations. The SCN score was determined using naturally infested field soil samples (Chang et al., 1997). Comparisons of trait and marker data were made by the F-test in analysis of variance (ANOVA) done with SAS (SAS Institute Inc., Cary, NC, 1988). Mapmaker-EXP 3.0 (Lander et al., 1987) was used to calculate map distances (cM, Haldane units) between linked markers and to construct a linkage map including traits as genes.

Results and Discussion

The ExF34 derived near isogenic line population was shown to be polymorphic in a region associated with resistance to SDS and SCN. A heterogenous region of about 10 cM encompassing Bng122D and $OI03_{450}$ but not extending to $OG13_{490}$ (Chang et al., 1997) or SATT163 (P. Cregan, personal communication) segregated within this population. Only three recombinants between Bng122D and $OI03_{450}$ were detected supporting the close linkage (2.2 cM) of these markers reported in RIL populations (Chang et al., 1996; 1997).

DNA Markers Associated With SDS Disease Index.

To detect loci conditioning partial resistance to SDS, we tested associations between $F_{5:9:13}$ genotypic classes for each DNA marker and the corresponding mean of square root DX (hereafter DX) across three locations over two years. Square root of DX was used as it provided the best fit to a bimodal distribution. Only one chromosomal region had significant ($P < 0.005$) effects on DX (Table 1). The region identified on linkage group G reported previously (Chang et al., 1996; 1997). In the ExF34 derived population the locus identified by RAPD marker $OI03_{450}$, accounted for 38% of total variation in mean DX, with $P = 0.0006$ (Table 1). The association was significant ($P \leq 0.001$) in all three locations. The RFLP marker Bng122D was also associated with mean DX ($R^2 = 0.38$, $P = 0.006$). Therefore, in the ExF34 derived population the QTL for resistance to SDS predicted to be closely linked to Bng122D and $OI03_{450}$ in RIL populations (Chang et al., 1996; 1997) was segregating in a second test population.

DNA Markers Associated With Resistance to Cyst Nematode race 3.

Associations between markers and SCN score were made with untransformed data by ANOVA. Only one chromosomal region had significant ($P < 0.005$) effects on resistance to SCN (Table 1). The region identified on linkage group G reported previously (Chang et al., 1996; 1997). In the ExF34 derived population the locus identified by RAPD marker $OI03_{450}$, accounted for 30% of total variation in mean SCN score in SCN infested soil, with $P = 0.003$ (Table 1). The RFLP marker Bng122D was also associated with mean SCN score ($R^2 = 0.23$, $P = 0.05$). Therefore, in the ExF34 derived population the QTL for resistance to SCN predicted to be closely linked to Bng122D and $OI03_{450}$ in a RIL population (Chang et al., 1996; 1997) was segregating in a second test population.

Mapping the QTL for disease resistance as qualitative markers

The discontinuous distribution of the SDS DX and SCN score suggested that separation of genotypes into resistance classes was valid for many genotypes for each trait (Table 2). We selected mean separations based on the LSD 0.01, that are appropriate for these small populations, and evidence of discontinuity in the populations. Three recombinants are evident within the forty lines of the ExF 34 derived population indicating that trait loci and marker loci are closely linked (Table 2). The marker order shown in the table avoids double-recombinants and is the most likely order. However, distances and orders are preliminary due to the small size of the population and potential for errors in the trait data.

Implications for breeding resistance to SDS

We have shown that the cluster of QTL underlying resistance to SDS and resistance to SCN race 3 can be isolated in near isogenic line populations. Within these populations the QTL behave as qualitative genes and accordingly may be assigned gene symbols after tests for allelism (Cregan et al., 1995). Recombinants between DNA markers and SDS and SCN resistance suggest the traits are encoded by separate loci that may correspond to *rhg1* (Webb et al., 1995; Concibido et al., 1996) and *rfs1* (Torto et al., 1996). The NIL populations are being enlarged and saturated with markers by AFLP (Meksem and Lightfoot, unpublished). Saturation mapping will allow unequivocal separation of *rhg1* and *rfs1*, dissection of each locus structure and ultimately lead to the isolation and DNA sequence determination of genes providing resistance to agriculturally important plant root pathogens.

Acknowledgments

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Table 1: Markers associated with disease resistance in the ExF34 derived NIL population.

	Marker					
	Bng122D			$OIO3_{450}$		
Trait	*P*	R^2	Allele±SEM	*P*	R^2	Allele±SEM
SCN	0.05	23%	E 0.44±0.19	0.006	30%	E 0.55±0.12
			F 0.19±0.18			F 0.24±0.11
SQRTDX	0.0006	38%	E 3.48±0.36	0.0006	38%	E 3.73±0.27
			F 2.09±0.34			F 2.54±0.2

SCN score, rating on a scale from 0 to 5 for SCN race 3 resistance. SQRTDX is the square root of disease index at R6 stage; DX is DI*DS/9. E is the allele derived from Essex, F is the allele derived from Forrest.

Table 2: Recombinant lines among ExF 34 derived population either resistant or susceptible based on marker and disease scores.

	$OI03_{450}$	SCN		SDS		Bng122D
Line	Allele	Score	Allele	DX Allele		Allele
3	F	0.23	F	4.8	E	E
17	F	0.48	E	4.2	E	E
23	F	0.00	F	1.2	F	E

An SCN score of less than 0.23 and more than 0.46 were the critical scores used to distinguish between resistant and susceptible lines when analyzing SCN score. A square root DX of less than 2.3 and more than 3.9 were the critical values used to distinguish between resistant and susceptible lines for disease index.

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A Second Tissue Culture-Induced Wrinkled-Leaf Mutation is Also Maternally Inherited

Introduction

We have found several wrinkled-leaf phenotypes in soybean plants regenerated from tissue cultures or protoplasts. The first one was identified in a plant regenerated via the organogenic regeneration system of Barwale et al. (1986) with cv. Asgrow A3127. Reciprocal crosses showed this trait to be maternally inherited (Stephen et al., 1991). This mutation apparently arose due to somaclonal variation and is one of a large number of mutants that have been described in regenerated soybean plants (summarized by Widholm, 1996).

Results

Another wrinkled-leaf phenotype was found in progeny of Clark 63 plants regenerated from protoplasts by the method of Dhir et al. (1991). Reciprocal crosses were made between the wrinkled-leaf phenotype that had the normal Clark 63 black hilum and with a Clark 63 isoline with yellow hilum. All F1s had imperfect black hilum color as expected. All F1 plants had wrinkled leaves if the female parent had wrinkled leaves while the F1 plants had normal leaves if the male parent had wrinkled leaves, thus indicating maternal inheritance.

Conclusions

Thus we conclude that the wrinkled-leaf mutation is maternally inherited in the two wrinkled-leaf mutants that we have studied genetically. This should mean that the mutation is carried by either the mitochondrial or plastid genome. No molecular studies have been carried out to confirm this however.

Acknowledgments

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Cryostorage of Embryogenic Soybean Suspension Cultures in Mineral Oil Following Preculture Treatments

Introduction

Soybean somatic embryos in suspension culture were first induced, cultured and regenerated into plants by Christianson et al. (1983), and a more reproducible method was described by Finer and Nagasawa (1988). Such cultures have been maintained in many laboratories for biochemical studies, selection work, and genetic transformation. However, the task of maintaining and initiating these soybean embryogenic suspension cultures, as with those of many other plant species, is tedious, time consuming, and expensive. The need for subculturing at regular intervals runs the risk of microbial contamination and the cultures many change genetically and lose the ability to regenerate plants, and in the case of soybean the plants regenerated may be infertile. To overcome these problems we have developed methods to preserve soybean somatic embryos in liquid nitrogen so cultures can be stored and recovered when needed.

Materials and Methods

Embryogenic suspension cultures of the *Glycine max* cv. Jack were established as described by Finer and Nagasawa (1988). After several months in culture, samples were used about 10d after being transferred to fresh Murashige and Skoog modified liquid medium with 5 mg/1 2,4-D and 15 mM glutamine (FG). Following various treatments, the embryogenic clumps were placed with a forceps into 2 ml cryovials (Corning Glass Works) and then frozen either directly in liquid nitrogen or after slow programmed cooling at the rate of 0.6 to 1°C/min to -40°C as described by Hauptmann and Widholm (1982). The tubes were kept at -40°C for 1h and then placed in liquid nitrogen. The tubes were thawed in a 40°C water bath.

Plants were regenerated as described by Finer and McMullen (1991).

Results and Discussion

To save space, only conditions that resulted in the recovery of viable growing cultures will be described. We were first able to recover growing cultures after cryostorage when a preculture treatment in FG medium with 0.1 mM abscisic acid (ABA) and 18.3 μM kinetin for 7d was used. The embryogenic cell clumps were then placed inside Ca alginate beads made by pipetting drops of 3% Na alginate (w/v) into 0.2% $CaCl_2$. The encapsulated clumps were then placed in FG medium with 17.7% sorbitol, 7.3% glycerol and 33.3% sucrose (Medium A) or 17.7% sorbitol, 7.3% glycerol, and 66.6% sucrose (Medium B) at one-half concentrations for 1 h and then overnight in full strength at 4°C in the dark with shaking. The clumps were blotted and dried in a laminar flow hood for 4 or 6h and then frozen in cryotubes without any liquid, 10 clumps per tube, by plunging into liquid nitrogen. Following thawing, the clumps were incubated in full and then half-strength medium for 1h each without shaking and then into FG. The cultures looked as if they were dead but by 20d green sections began to grow. Twenty plants were regenerated from these cultures, all of which were sterile, probably because the culture was too old.

Growing cultures were also recovered if precultured as above and then air dried for 0.5 to 2h in the hood without encapsulation. About 30 clumps were then placed in each cryotube with 1 ml mineral oil and frozen directly in liquid nitrogen. About 70% of the clumps survived after thawing and 15 plants were regenerated. All 5 plants that were grown to maturity set seed.

The cultures were also precultured in Medium A or B or in half-strength concentrations as shown in Table 1. Successful recovery of growing cultures from about two-thirds of the clumps that were capable of regenerating fertile plants was attained with several combinations of preculture media, times, temperature, cooling rate and recovery media all with the embryogenic clumps being placed in cryotubes with 1.2 ml mineral oil before freezing.

We have been able to successfully cryopreserve embryogenic soybean suspension cultures using a number of different preculture, encapsulation, drying, freezing, and recovery conditions. The simplest protocol is to preculture for 3d at 4°C with shaking in medium A or B, place the clumps in mineral oil in cryotubes and plunge directly in liquid nitrogen. The

tubes are then thawed in a 40°C water bath and clumps placed directly into FG medium with 26% sucrose on a shaker before transfer to FG medium after 14d.

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Table 1. Successful preculture, freezing, and recovery conditions

Preculture media, time and temp.	Freezing rate	Recovery solution
A 3d 4°C	rapid	FG + 26% sucrose
A 3d 4°C	slow	FG + 26% sucrose
B 3d 4°C	slow	FG
B 3d 4°C	rapid	FG + 26% sucrose
½B 1d, B 2d 4°C	rapid	FG + 26% sucrose
½B 1d, B 2d 28°C	slow	FG + 26% sucrose
½B 1d, B 2d 28°C	slow	FG

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Allelism of a Necrotic Root Mutant in *Glycine max*

Abstract

Disease mimic mutations have been identified in several plant species. To date, however, only leaf disease mimics have been reported. In soybean, *Glycine max* L. Merr., three root disease mimics (necrotic root) have been identified. The objective of this experiment was to determine possible allelism of an unknown necrotic root mutation identified among progeny of germinal revertants of *w4-m*. Normal plants from families segregating normal and necrotic root phenotypes were crossed to known necrotic root heterozygotes to determine if the unknown was an allele of the necrotic root mutants, Genetic Types T328H, T329H, and T330H. Both F_1 and F_2 data support the conclusion that the unknown necrotic root mutation was an allele of the three known mutants.

Introduction

Previous experiments have identified three recessive, allelic, necrotic root mutants descended from germinal revertants in the *w4*-mutable line of *Glycine max* (L.) Merr. (Kosslak et al. 1996). These mutants arose from separate genetic events and were designated NR-1, NR-2, and NR-3 (Palmer et al. 1989). These mutations have been given the gene symbols *Rn rn* (Ames 1) =T328H, *Rn rn* (Ames 2) =T329H, and *Rn rn* (Ames 3) =T330H. A transposable element has been suggested as the cause of the *w4-m* allele instability (Groose et al. 1988, 1990; Palmer et al. 1989; Palmer and Groose 1993).

In another gene tagging study involving the *w4-m* allele, an unknown mutant was identified which expressed a similar necrotic root phenotype to the three previous necrotic root mutations. The purpose of this experiment was to determine if the unknown mutant was an allele of the previously discovered loci, or an independent disease lesion mimic of another gene.

Materials and Methods

The unknown necrotic root mutant was isolated in a gene tagging study involving the *w4-m* allele. Normal plants from families segregating normal and necrotic root phenotypes were crossed to heterozygotes of the three previously discovered necrotic root mutants, NR-1, NR-2, and NR-3. The known necrotic root mutants were found in the same line, but in an independent gene tagging study. The normal F_1 plants were assigned identification numbers and allowed to self-pollinate. The F_2 seed were planted on germination paper, and labeled to maintain family identity. The F_2 seedlings were scored for root necrosis seven days after germination.

The F_2 families were scored as either segregating the necrotic root phenotype, or nonsegregating. When viewed under UV light, the areas of root necrosis were brown and nonflourescent. Normal plants showed root fluorescence characteristic of their genetic background. This served as an effective phenotype for scoring. The results were analyzed for statistical significance by using a chi-square test.

Results and Discussion

The data collected from the crosses between the unknown heterozygote and the known heterozygote *Rn rn* (Ames 1) =T328H, indicated an allelic relationship (Table 1). The F_1 plants displayed the characteristic 3:1 ratio of expected dominant nonmutant : recessive necrotic root mutant plants. The F_2 families segregated in the expected 2:1 Mendelian ratio. Within segregating families, F_2 plants displayed the 3:1 ratio of nonmutant : necrotic root mutants. The data collected from the crosses between the unknown heterozygote and the heterozygote *Rn rn* (Ames 2) =T329H, also indicated an allelic relationship. The F_1 plants displayed the expected 3:1 ratio of nonmutant : necrotic root mutant plants. The F_2 families segregated in the expected 2:1 Mendelian ratio. Within segregating families, F_2 plants also displayed the 3:1 ratio of nonmutant : necrotic root mutants. The data collected from the crosses between the unknown heterozygote and the known heterozygote *Rn rn* (Ames 3) =T330H, also indicated an allelic relationship. The F_1 plants displayed the characteristic 3:1 ratio of nonmutant : necrotic root mutant plants, also found in the other crosses. F_2 families segregated in the expected 2:1 Mendelian ratio. Within segregating families, the F_2 plants displayed the 3:1 ratio of nonmutant : necrotic root

mutants. The unknown has thus been concluded to be allelic with the three previously described necrotic root mutants, as shown by nonsignificant P values.

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Table 1. Allelism tests of unknown necrotic root mutant with three known necrotic root mutants in soybean; F_1 and F_2 data.

	No. F_1 plants				No. F_2 families				No. F_2 plants			
Cross	Non-mutant	Necrotic	X^2(3:1)	P	Seg.	Nonseg.	X^2(2:1)	P	Non-mutant	Necrotic	X^2(3:1)	P
Unknown (het.) X *Rn rn* (Ames 1)	75	36	3.27	0.07	17	11	0.45	0.51	538	187	0.24	0.64
Unknown (het.) X *Rn rn* (Ames 2)	37	12	0.007	0.94	14	11	1.28	0.26	529	174	0.02	0.89
Unknown (het.) X *Rn rn* (Ames 3)	33	9	0.28	0.62	14	10	0.75	0.40	574	161	3.76	0.05

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Genetic Analysis of Root Fluorescence of Central Chinese Germplasm

Root fluorescence is a phenomenon in which roots of seedlings fluoresce when irradiated with ultraviolet light. Four recessive loci, fr_1, fr_2, fr_4, and fr_5 and one dominant locus Fr_3 condition nonfluorescent roots in soybean (Delannay and Palmer, 1982; Sawada and Palmer, 1987).

In 1992, 500 accessions were received from China representing nine provinces in North China. Previous accessions from these regions numbered 172. These 500 accessions were classified into 736 accessions after agronomic and descriptive evaluations were completed.

Our objectives were to screen the 736 accessions for the presence of fluorescent and nonfluorescent roots, and determine the genetics of any identified nonfluorescent root accessions. In addition, the 32 nonfluorescent root accessions that were identified were classified for seed coat peroxidase and pubescence tip.

Materials and Methods

About 10 seed from each of the 736 accessions were germinated on paper towels and the 3-day-old seedlings were examined under an ultraviolet light. Remnant seed of nonfluorescent accessions were planted at the Bruner Farm near Ames, Iowa. Cross-pollinations were made between nonfluorescent accessions and the fluorescent root cultivars BSR 101 or Harosoy. Cross-pollinations were made between nonfluorescent accessions and two nonfluorescent testers, near isogenic lines Hark-fr_1 and Hark-fr_2. Fifty-nine accessions out of 572 accessions were identified previously that were nonfluorescent roots and 41 were fr_1fr_1 and 15 were fr_2fr_2 genotype (Delannay and Palmer, 1982). Thus cross-pollinations for allelism with the Central Chinese germplasm were made only with the fr_1fr_1 and fr_2fr_2 testers.

Seed coat peroxidase was scored on four seed from each of the 32 nonfluorescent root accessions by using the test suggested by Buzzell and Buttery (1969). Pubescence tip was classified as sharp-tipped trichomes or blunt-tipped trichomes (Ting 1946).

Results and Discussion

A total of 32 accessions out of 736 were identified as nonfluorescent root accessions (Table 1). The 32 nonfluorescent root accessions were inherited as single recessive genes. Allelism tests with Hark-fr_1 and Hark-fr_2 testers indicated that 30 accessions were fr_1fr_1 genotype and only 2 accessions were fr_2fr_2 genotype (Table 1).

Of the 30 fr_1fr_1 accessions, only one was from Shandong province, 14 from Gansu province, and 15 from Shanxi province (Table 2). The two fr_2fr_2 accessions were from Shanxi province (Table 2). Delannay and Palmer (1982) reported 7.18% fr_1fr_1 and 2.62% fr_2fr_2 from Asian and European accessions while the Chinese accessions were 4.08% fr_1fr_1 and 0.27% fr_2fr_2.

Delannay and Palmer (1982) reported that all 15 of the 59 nonfluorescent root accessions that were fr_2fr_2 originated in Europe and that they had blunt pubescent tip. The two accessions from Shanxi province that were fr_2fr_2 are the first fr_2fr_2 mutants reported from China. Of the 32 nonfluorescent mutants, 27 were sharp pubescent tip while only 5 were blunt pubescent tip, which included the two fr_2fr_2 accessions (Table 3). Thirteen nonfluorescent root mutants had been identified after mutagen treatment and five were allelic to the fr_2fr_2 tester (Sawada and Palmer, 1987).

The data on seed coat peroxidase activity indicated that 15 accessions were high activity, 16 accessions were low activity, and that one accession (PI 567.466B) was a mixture (Table 3).

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Table 1. F_2 segregation of nonfluorescent root of Central Chinese accessions crossed with wildtype plants and with Hark-*fr*$_1$ and Hark-*fr*$_2$ testers.

PI number	Crosses with wild type					Crosses with *fr*$_1$*fr*$_1$ tester					Crosses with *fr*$_2$*fr*$_2$ tester				
	F_1	No. F_2 plants		$\chi^2_{(3:1)}$	P	F_1	No. F_2 plants		$\chi^2_{(9:7)}$	P	F_1	No. F_2 plants		$\chi^2_{(9:7)}$	P
		F	NF				F	NF				F	NF		
567.292	2	205	64	0.21	0.66	10	0	299			9	152	117	0.01	0.93
567.294	3	321	102	0.18	0.68	7	0	210			6	94	75	0.03	0.88
567.297	2	223	88	1.80	0.19	7	0	203			1	83	61	0.11	0.76
567.299B	3	299	111	0.94	0.34	10	0	293			5	86	64	0.07	0.82
567.304	4	431	137	0.24	0.64	4	0	153			7	117	91	0	1.00
567.305	2	198	78	1.56	0.21	14	0	417			8	129	103	0.04	0.86
567.314	2	287	104	0.53	0.48	8	0	263			6	140	103	0.18	0.68
567.322	3	330	117	0.33	0.58	8	0	220			10	153	145	2.92	0.09
567.327	4	429	148	0.13	0.73	9	0	270			10	145	136	2.47	0.11
567.330	5	495	169	0.07	0.82	10	0	298			9	156	109	0.74	0.41
567.332	2	198	60	0.46	0.50	8	0	208			2	99	77	0	1.00
567.336A	2	237	65	1.95	0.17	5	0	150			5	85	64	0.04	0.86
567.336B	2	249	77	0.33	0.58	5	0	149			5	73	62	0.26	0.63
567.342	4	445	140	0.36	0.56	6	0	179			6	95	81	0.37	0.56
567.348	3	298	95	0.14	0.71	10	0	298			8	135	102	0.05	0.85
567.353	2	269	71	3.07	0.08	9	0	256			9	149	111	0.12	0.74
567.417C	4	437	145	0.002	0.98	4	0	120			4	80	58	0.17	0.69
567.419A	3	329	125	1.55	0.22	6	0	175			5	104	83	0.03	0.88
567.420	2	208	72	0.08	0.80	10	0	297			10	171	126	0.21	0.66
567.421	2	217	77	0.22	0.65	10	0	298			7	4	137	0.46	0.50
567.423	2	300	106	0.27	0.62	10	0	297			9	144	117	0.12	0.74
567.428	4	489	169	0.16	0.69	9	0	265			10	164	129	0.01	0.93
567.436	3	350	132	1.46	0.23	5	0	384			6	99	68	0.62	0.45
567.440	2	245	78	0.12	0.74	11	0	319			10	160	125	0.00	0.99
567.441A	2	197	68	0.06	0.83	5	0	147			3	67	50	0.05	0.85
567.446	2	283	79	1.95	0.17	3	96	78	0.08	0.80	8	0	236		
567.459	3	339	108	0.17	0.69	6	0	180			9	148	119	0.07	0.82
567.462	3	292	111	1.39	0.24	8	135	102	0.04	0.86	6	0	174		
567.466A	3	380	120	0.27	0.62	5	0	150			4	97	81	0.22	0.65
567.466B	2	208	75	0.34	0.58	5	0	149			3	73	61	0.17	0.69
567.467	4	444	133	1.17	0.28	9	0	269			5	83	62	0.06	0.83

Table 2. Total number of accessions and nonfluorescent root accessions per province.

Province	Total	No. nonfluorescent	
		fr$_1$*fr*$_1$	*fr*$_2$*fr*$_2$
Anhui	67	0	0
Gansu	103	14	0
Hebei	50	0	0
Henan	112	0	0
Jiangsu	76	0	0
Ningxia	25	0	0
Shaanxi	67	0	0
Shandong	141	1	0
Shanxi	95	15	2
	736	30	2

% *fr*$_1$*fr*$_1$ = 4.08
% *fr*$_2$*fr*$_2$ = 0.27

Table 3. Pubescence tip and seed coat peroxidase activity classification of the 32 nonfluorescent soybean accessions.

PI number	Pubescence tip		Seed coat peroxidase activity	
567.287	Sharp			Low
567.292	Sharp		High	
567.294	Sharp			Low
567.297		Blunt		Low
567.299B	Sharp		High	
567.304	Sharp		High	
567.305	Sharp		High	
567.314	Sharp		High	
567.322	Sharp			Low
567.327	Sharp			Low
567.330	Sharp			Low
567.332	Sharp			Low
567.336A	Sharp		High	
567.336B	Sharp		High	
567.342	Sharp		High	
567.348	Sharp			Low
567.353	Sharp			Low
567.417C		Blunt		Low
567.419A	Sharp		High	
567.420	Sharp			Low
567.421	Sharp			Low
567.423	Sharp			Low
567.428	Sharp		High	
567.436	Sharp		High	
567.440	Sharp			Low
567.441A	Sharp		High	
567.446		Blunt	High	
567.459	Sharp			Low
567.462		Blunt	High	
567.466A	Sharp		High	
567.466B*	Sharp		High	Low
567.467		Blunt		Low

* PI 567.466B was a mixture of high and low seed coat peroxidase activity

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Genetic Characterization of a Duplicate-Factor Male-sterile, Female-sterile Trait in Soybean

Introduction

The k_2 (tan saddle seed coat), *Mdh1-n* (mitochondrial malate dehydrogenase 1 null), and y_{20} (yellow foliage) chromosome region in soybean has been of great interest to us (Palmer, 1984; Chen, 1996). One focus has been to study recombination both in coupling and repulsion phases. In the cross of L67-3843 (k_2 *Mdh1* Y_{20}) x T323 (K_2 *Mdh1-n* y_{20}), a ratio of 98 (fertile + partial-sterile): 6 completely sterile plants was observed in the F_2 generation. The genetic characterization of the partial-sterile phenotype from L67-3843 is reported separately (Palmer, 1997).

Our objective was to characterize genetically the male-sterile, female-sterile trait found in the F_2 generation of the cross L67-3843 x T323.

Materials and Methods

Reciprocal cross-pollinations were made between L67-3843 and T323. All the F_1 plants were partial female-sterile as determined phenotypically by pod set. Pollen samples were collected from four F_2 families, stained with I_2KI, and examined microscopically. All sampled plants from three F_2 families had well-stained red-brown and plump pollen grains, characteristic of fertile (viable) pollen. In family A93-B-277 (L67-3843 x T323), 2 of 10 F_2 plants had aborted pollen grains. At maturity these two plants bore no pods. This F_2 family segregated 98 partial-sterile plants plus normal (fertile) plants and 6 completely sterile plants. The 98:6 ratio suggested duplicate-factor inheritance, $\chi^2_{(15:1)} = 0.04$. F_2 plants were single-plant threshed and the $F_{2:3}$ progenies were classified for fertility/sterility.

In $F_{2:3}$ progenies segregating approximately 3 fertile: 1 completely-sterile plant, 25 fertile plants were single-plant threshed and the $F_{3:4}$ progenies were classified for fertility/sterility. In the $F_{2:3}$ progenies segregating approximately 15 fertile: 1 completely-sterile plant, 55 fertile plants were single-plant threshed and the $F_{3:4}$ progenies were classified for fertility/sterility.

In $F_{3:4}$ progenies segregating approximately 3 fertile: 1 completely-sterile plant, 12 fertile plants from each of six families were single-plant threshed and the $F_{4:5}$ progenies were classified for fertility/sterility. The six families were used in the following genetic tests to identify the two different loci of the duplicate-factor male-sterile, female-sterile phenotype.

In the six families segregating 3 fertile: 1 sterile plant, eight fertile plants per family were numbered and the identification number was maintained during cross-pollination. About 16 cross-pollinations (including reciprocals) were made for each combination such that each family was crossed to the other five families for the allelism test to identify which loci were allelic, or conversely which loci were nonallelic. The numbered plants were progeny tested to distinguish homozygous dominant genotypes from heterozygous genotypes.

Table 1 presents the theoretical values that are expected when identical genotypes for the 3 fertile: 1 sterile segregation are intercrossed. Table 2 presents the theoretical values that are expected when different genotypes are intercrossed.

Results

Sixty-two fertile F_2 plants, from the 98 fertile: 6 sterile segregation from the cross of L67-3843 x T323, were single-plant threshed and evaluated in the $F_{2:3}$. The expected 7 (nonsegregating): 4 (3 fertile : 1 sterile) : 4 (15 fertile : 1 sterile) ratio was observed (Table 3). The $F_{3:4}$ and $F_{4:5}$ generations segregated as expected, on a family basis and for the number of fertile:sterile plants, for a duplicate-factor inherited trait (Tables 3 and 4).

Based upon the F_2 segregation of the intercrosses, the six families could be separated into two different genotypic classes (Aabb) and (aaBb). Three families originated from Aabb and three families originated from aaBb.

The 48 plants (6 families x 8 plants/family) selected for the intercrosses to identify the separate 3:1 segregation for the duplicate factor test for sterility at the A locus gave 7:17 (homozygous dominant: heterozygous) plants for the AAbb: Aabb genotypes, respectively. At the B locus, 8:16 (homozygous dominant: heterozygous) plants were observed for the aaBB:aaBb genotypes, respectively. The observed segregation for the two different genotypic classes was 24:24, $\chi^2_{1:1} = 0$, $P = 1.00$, and the observed segregation for the homozygous

dominant : heterozygous genotypes was 15:33, $\chi^2_{1:2}$ = 0.09, P = 0.76.

The intercrosses between families of identical genotype gave 27 fertile: 4 sterile F_1 plants ($\chi^2_{8:1}$ = 1.0, P = 0.32). The F_2 family segregation for nonsegregating : (3:1) from cross-pollinations of homozygous dominant x heterozygous or reciprocal, fit the expected 1:1 ratio (Table 5). And the F_2 family segregation for nonsegregating : (3:1) from cross-pollinations of heterozygous x heterozygous genotypes fit the expected 1:2 ratio (Table 5). Within segregating families both cross-pollination combinations fit the expected 3 fertile : 1 sterile ratio (Table 6).

The intercrosses between families of different genotypes gave 73 fertile : 10 sterile F_1 plants ($\chi^2_{8:1}$ = 0.07, P = 0.79). The F_2 family segregation for 3:1 to 15:1 from cross-pollinations of homozygous dominant x heterozygous or reciprocal fit the expected 1:1 ratio (Table 7). And the F_2 family segregation for 3:1 to 15:1 from cross-pollinations of heterozygous x heterozygous genotypes fit the expected 1:2 ratio (Table 7). Within segregating families all cross-pollination combinations fit the expected 3:1 or 15:1 ratios (Table 8).

Discussion

Four F_2 families from the cross of L67-3843 x T323 were sampled for pollen analysis to determine if the partial-sterility trait transmitted by L67-3843 could be detected by pollen analysis. Three F_2 families gave all male-fertile plants as judged by I_2KI staining yet ovule abortion or very early embryo abortion was observed in about half of the plants (Palmer, 1997). The fourth F_2 family gave 8 fertile : 2 completely sterile plants as judged by I_2KI staining. At maturity this family (A93-B-277), had 98 partial-sterile plants plus normal (fertile) plants : 6 sterile plants.

Our hypothesis was that we had a duplicate - factor trait for male sterility, female sterility. The most plausible explanation was that a mutation occurred in the F_1 plant from A to a. One parent was BB and the other parent bb and both parents were AA, so that the F_1 plant (with the A to a mutation) was AaBb and upon self-pollination segregated 15:1 in the F_2 generation. The F_3 generation confirmed the duplicate-factor inheritance pattern.

Eight families that segregated approximately 3 fertile : 1 sterile were selected in the F_4 generation and were used in cross-pollinations in an allelism test to distinguish the two different loci that contribute to the duplicate-factor inheritance. The theoretical expectation was that four families were homozygous recessive at the first locus and heterozygous at the second locus and that the other four families were heterozygous at the first locus and homozygous recessive at the second locus. The F_2 segregation data confirmed that the actual ratio was 4:4.

The F_1 phenotypes are the same for intercrosses among identical genotypes and among different genotypes. The F_2 segregation distinguishes between the two genotypic classes. Crosses between identical genotypes produced all-fertile F_2 families and families that segregated 3:1. Crosses between different genotypes produced segregating families in equal frequency; 3:1 and 15:1 ($\chi^2_{1:1}$ = 3.26, P = 0.07). Within segregating families of both identical and different genotype combinations, χ^2 values were nonsignificant. The F_2 data confirmed that two different genotypes had been identified.

All the F_2 families analyzed to identify the two different loci of the duplicate-factor phenotype were derived from parents of known genotypes, i.e. each parent was confirmed by progeny testing as either homozygous dominant or heterozygous. Additional F_1 plants where only the male-parent genotype was identified by progeny testing have been advanced to the F_2 generation. Several hundred F_2 families will be classified for fertility/sterility at maturity in fall 1997. These additional data are expected to strengthen our conclusions based upon this smaller F_2 population.

Allelism tests of these two different male-sterile, female-sterile loci will be made with the known mutants; st_2, st_3, st_4, st_5, and st_6st_7. The T331 ($st_6st_6st_7st_7$) mutant also is male-sterile, female-sterile but no attempt was made to identify the separate loci. After the allelism tests are complete, gene symbols will be assigned to the male-sterile, female-sterile mutant identified in the F_2 generation from the cross of L67-3843 x T323.

Soybean is considered a diploidized polyploid. Zobel (1983) estimated that the soybean [*Glycine max* (L.) Merr.] genome is 82% duplicated. The number of traditional (classical) Mendelian markers exhibiting duplicate-factor inheritance is about 33% (Palmer and Kilen, 1987). As more detailed studies with classical Mendelian markers are made, additional duplicate factors (Ilarslan et al., 1997) or more complex rearrangements have been identified (Palmer, 1984; Chen, 1996). Shoemaker et al. (1996), using molecular markers, suggested that more than 90% of the nonrepetitive sequences in soybean may be present in two or more copies. Furthermore, Shoemaker et al. (1996) believe that the presence of nested duplications indicates that at

least one of the original genomes may have undergone an additional round of tetraploidization.

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Table 1. Theoretical expectations of intercrosses among fertile plants from families segregating 3 fertile:1 sterile plant; identical genotypes.

Parent 1[a]	Genotypic frequency	Phenotype	Parent 2[a]	Genotypic frequency	Phenotype
AAbb	1	Fertile	AAbb	1	Fertile
Aabb	2	Fertile	Aabb	2	Fertile
aabb	1	Sterile	aabb	1	Sterile

Cross-pollination combinations	F_1 frequency and phenotype	F_2 frequency and F_3 phenotype
AAbb x AAbb	all fertile	all fertile
AAbb x Aabb	all fertile	1 (all fertile):1 (3 fertile:1 sterile)
Aabb x AAbb	all fertile	1 (all fertile):1 (3 fertile:1 sterile)
Aabb x Aabb	3 fertile:1 sterile	1 (all fertile):2 (3 fertile:1 sterile)

[a] Identical results are expected in the F_1 and F_2 generation, if the crosses are aaBB, aaBb x aaBB, aaBb and if the crosses occur in equal frequencies and if loci A and B are not linked.

Table 2. Theoretical expectations of intercrosses among fertile plants from families segregating 3 fertile:1 sterile plant; different genotypes.

Parent 1	Genotypic frequency	Phenotype	Parent 2	Genotypic frequency	Phenotype
AAbb	1	Fertile	aaBB	1	Fertile
Aabb	2	Fertile	aaBb	2	Fertile
aabb	1	Sterile	aabb	1	Sterile

Cross-pollination combinations	F_1 frequency and phenotype	F_2 frequency and F_3 phenotype
AAbb x aaBB	All fertile	all (15 fertile:1 sterile)
AAbb x aaBb	All fertile	1 (3 fertile:1 sterile):1 (15 fertile:1 sterile)
Aabb x aaBB	All fertile	1 (3 fertile:1 sterile):1 (15 fertile:1 sterile)
Aabb x aaBb	3 fertile:1 sterile	2 (3 fertile:1 sterile):1 (15 fertile:1 sterile)

Table 3. Number of F_2 , F_3, and F_4 families that were all fertile plants, 3:1 segregation, or 15:1 segregation from the cross of L67-3483 x T323.

	Segregation patterns and number of families						
Generation	NS	3:1	15:1	$\chi^2_{(7:4:4)}$	P	$\chi^2_{(1:2)}$	P
$F_{2:3}$	31	17	14	0.17	0.92		
$F_{3:4}$ (from 3:1 segregation of $F_{2:3}$ families)	8	17				0.02	0.89
$F_{3:4}$ (from 15:1 segregation of $F_{2:3}$ families)	28	12	15	0.71	0.70		
$F_{4:5}$ (from 3:1 segregation of $F_{3:4}$ families)	28	44				1.00	0.32

Table 4. Number of fertile plants and completely-sterile plants in the F_2, F_3, and F_4 generations from the cross of L67-3483 x T323.

	Number of plants				Number of plants			
Generation	Fertile	Sterile	$\chi^2_{(3:1)}$	P	Fertile	Sterile	$\chi^2_{(15:1)}$	P
$F_{2:3}$	680	213	0.63	0.43	986	78	2.12	0.15
$F_{3:4}$ (from 3:1 segregation of $F_{2:3}$ families)	476	149	0.45	0.50				
$F_{3:4}$ (from 15:1 segregation of $F_{2:3}$ families)	233	69	0.75	0.39	676	46	0.02	0.89
$F_{4:5}$ (from 3:1 segregation of $F_{3:4}$ families)	1006	302	2.55	0.11				

Table 5. Number of F_2 families from intercrosses among fertile plants from families segregating 3 fertile:1 sterile plant; identical genotypes.

Cross-pollination combinations[a]	No. F_2 families		Within segregating combinations			
	All fertile	3:1	$\chi^2_{(1:1)}$ [b]	P	$\chi^2_{(1:2)}$ [c]	P
AAbb x AAbb	8	0				
(AAbb x Aabb / Aabb x AAbb)	8	5	0.69	0.41		
Aabb x Aabb	2	4			0	1.00

[a] Similar results are expected for number of F_2 families and within segregating combinations if the crosses are aaBB, aaBb x aaBB, aaBb and if the crosses occur in equal frequencies and if loci A and B are not linked.

[b] Expected F_2 frequency and F_3 phenotype; 1(all fertile):1 (3 fertile:1 sterile).

[c] Expected F_2 frequency and F_3 phenotype; 1(all fertile):2 (3 fertile:1 sterile).

Table 6. Number of F_2 plants within segregating combinations from intercrosses among fertile plants from families segregating 3 fertile:1 sterile plant; identical genotypes.

Cross-pollination combinations[a]	No. F_2 plants			
	Fertile	Sterile	$\chi^2_{(3:1)}$	P
(AAbb x Aabb / Aabb x AAbb)	467	150	0.16	0.69
Aabb x Aabb	365	110	0.86	0.35

[a] Similar results are expected for number of F_2 plants if the crosses are aaBB, aaBb x aaBB, aaBb and if the crosses occur in equal frequencies and if loci A and B are not linked.

Table 7. Number of F_2 families from intercrosses among fertile plants from families segregating 3 fertile:1 sterile plant; different genotypes.

Cross-pollination combinations	No. F_2 families			Within segregating combinations			
	All fertile	3:1	15:1	$\chi^2_{(1:1)}$ [a]	P	$\chi^2_{(2:1)}$ [b]	P
AAbb x aaBB	0	0	4				
(AAbb x aaBb / Aabb x aaBB)	0	18	12	1.20	0.27		
Aabb x aaBb	0	24	15			0.62	0.43

[a] Expected F_2 frequency and F_3 phenotype; 1(3 fertile:1 sterile):1 (15 fertile:1 sterile).

[b] Expected F_2 frequency and F_3 phenotype; 2(3 fertile:1 sterile):1 (15 fertile:1 sterile).

Table 8. Number of F_2 plants within segregating combinations from intercrosses among fertile plants from families segregating 3 fertile:1 sterile plant; different genotypes.

Cross-pollination combinations	No. F_2 plants				No. F_2 plants			
	Fertile	Sterile	$\chi^2_{(3:1)}$	P	Fertile	Sterile	$\chi^2_{(15:1)}$	P
AAbb x aaBB					769	55	0.25	
(AAbb x aaBb / Aabb x aaBB)	1520	498	0.11	0.74	1506	100	0.001	0.99
Aabb x aaBb	1932	652	0.07	0.79	1949	135	0.18	0.67

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Genetic Characterization of a Partial-Female Sterile from L67-3483

Introduction

L67-3483 was identified in 1956 at Columbia, Missouri in the cultivar Clark after x-ray radiation. L67-3483 has tan-saddle seed coat phenotype (*K_2* locus) and is a near-isogenic line of Clark (Rode and Bernard, 1975).

It was noticed that in reciprocal cross-pollinations of L67-3483 with cultivar Minsoy, that all F_1 plants had many one- and two-seeded pods. The abortions appeared to be ovule abortions or very early embryo abortions phenotypically similar to ovule abortions in heterozygous chromosome translocation plants (Palmer and Heer, 1984). Pollen samples were collected from F_2 plants of reciprocal crosses of L67-3483 x Minsoy, stained with I_2KI, and examined microscopically. All pollen grains from all plants (greater than 200) were well-stained red brown, and plump, characteristic of fertile/viable pollen.

Our objective was to determine the genetics of the partial-female sterile trait in L67-3483.

Materials and Methods

Cultivar Clark (female parent) was crossed with L67-3483. All F_1 plants expressed partial female sterility. Sixteen fertile F_2 plants, progeny of a single F_1 plant, were identified at harvest, i.e. had normal pod set, and were single-plant threshed.

F_3 plants from progeny rows of these 16 fertile F_2 plants were crossed as female parent to cultivar BSR 101 and to L67-3483 at the University of Puerto Rico - Iowa State University Soybean Nursery near Isabela, Puerto Rico in March 1995. The F_1 plants were grown in the USDA glasshouse at Ames, Iowa in summer 1995. Pollen samples from each F_1 plant were collected, stained with I_2KI and examined microscopically. Seed set on each F_1 plant was classified phenotypically as normal or partial sterile. The F_1 plants were single-plant threshed and evaluated in the F_2 generation at the Bruner Farm near Ames, Iowa in summer 1996.

Results

Testcross progeny, of the 16 fertile F_2 plants from the Clark x L67-3483 crosses, gave 10 fertile: 6 partial-sterile plants from BSR 101 crosses (Table 1). The identical 16 fertile F_2 plants gave testcross progeny of 6 fertile: 10 partial-sterile plants when crossed to L67-3483 (Table 1). The F_1 data were confirmed by the F_2 data. All fertile (normal) F_1 plants were fertile (normal) plants in the F_2. All partial sterile F_1 plants segregated partial sterile and fertile (normal) plants in the F_2.

In our testcrosses with BSR 101 and L67-3483, we had an exact correspondence of genotype and phenotype. If the fertile F_2 plants (from the cross of Clark x L67-3843) gave a partial-sterile phenotype in the testcross with BSR 101, they gave a normal (fertile) phenotype in the testcross with L67-3843. Alternatively, if a normal (fertile) phenotype was observed in the testcross with BSR 101, a partial-sterile phenotype was evident with L67-3843. Thus the F_1 plants consisted of two genotypes in equal frequency (10:6, x^2 1:1 = 1.00). One genotype corresponded, as expected, to the genotype of L67-3843 used in the initial cross-pollination of Clark x L67-3843. The F_1 phenotypic classification was confirmed in the F_2 generation.

Discussion

The phenotype of L67-3843 is similar (?identical) to PS-1, PS-2, PS-3, and PS-4, four partial female-sterile mutants that had been identified from a gene-tagging study (Palmer et al., 1989). PS-1 has a different genetic inheritance pattern than PS-2, PS-3, and PS-4. The PS-1 mutant is a true breeding single gene recessive (Pereira, Lersten, and Palmer, in press). Pollen of the PS-1 plants is fertile (and considered viable) as measured by differential staining and germination tests. The PS-2, PS-3, and PS-4 mutants transmit the partial female-sterility phenotype only through the male parent (Pereira, Ilarslan, and Palmer, in press). That is, the phenotype is not transmitted when partial-sterile plants are used as female parents. When PS-2, PS-3, and PS-4 plants are used as male parents in cross-pollinations, the F_1 plants segregate in a 1 fertile: 1 partial sterile ratio. The partial-sterile phenotype is maintained by self-pollination of partial-sterile plants. Testcrosses of the male and female fertile plants gave all fertile F_1 and F_2 progenies (Pereira, Ilarslan, and Palmer, in press). Pollen of the PS-2, PS-3, and PS-4 fertile siblings is fertile (and considered viable) as measured by differential staining and germination tests.

The inheritance pattern of the partial female-sterility of L67-3843 is different than the pattern of PS-1, and PS-2, PS-3, and PS-4. L67-3843 can be propagated as a homozygote and the phenotype can be transmitted either via the male or the female parent in cross-pollinations. Also the phenotype can be transmitted by self-pollination of partial-sterile plants which are heterozygous for the trait. Both homozygous genotypes are male fertile and female fertile.

In soybean, five partial-female sterile mutants have been identified and represent three distinct inheritance patterns. The soybean mutants have a phenotype similar to the lethal ovule mutants reported in maize (Clark, 1942; Nelson and Clary, 1952; Singleton and Mangelsdorf, 1940; Van Horn and Nelson, 1969).

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Table 1. Testcrosses, of male fertile and female fertile F_2 plants from Clark x L67-3483, with BSR 101 and L67-3483 to identify chromosome structure of L67-3483; F_1 and F_2 data.

Plant no.	BSR 101 testcross			L67-3483 testcross		
	F_1 plants		F_2 plants	F_1 plants		F_2 plants
(A94)	No.	Seed set	Seed set	No.	Seed set	Seed set
B-4	8	Normal	Normal	4	Partial sterile	Partial sterile and normal
B-9	7	Partial sterile	Partial sterile and normal	4	Normal	Normal
B-10	9	Partial sterile	Partial sterile and normal	4	Normal	Normal
B-11	6	Normal	Normal	3	Partial sterile	Partial sterile and normal
B-29	7	Normal	Normal	4	Partial sterile	Partial sterile and normal
B-46	8	Normal	Normal	7	Partial sterile	Partial sterile and normal
B-53	7	Normal	Normal	4	Partial sterile	Partial sterile and normal
B-83	4	Normal	Normal	3	Partial sterile	Partial sterile and normal
B-88	3	Partial sterile	Partial sterile and normal	3	Normal	Normal
B-94	8	Normal	Normal	4	Partial sterile	Partial sterile and normal
B-106	4	Normal	Normal	3	Partial sterile	Partial sterile and normal
B-119	8	Normal	Normal	4	Partial sterile	Partial sterile and normal
B-128	6	Partial sterile	Partial sterile and normal	4	Normal	Normal
B-137	4	Partial sterile	Partial sterile and normal	2	Normal	Normal
B-146	6	Partial sterile	Partial sterile and normal	4	Normal	Normal
B-162	4	Normal	Normal	8	Partial sterile	Partial sterile and normal

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Genetic Study of a Diaphorase 2 null Mutant

Abstract

In a survey of Central Chinese soybean germplasm, PI 567565 was characterized as a *Dia2* null mutant. In the analysis of F2 seeds from the cross, BSR 101 x PI 567565, a 3 present to 1 null ratio pattern suggested that *Dia2-n* was a single recessive allele. No linkages between the *Dia2* locus and the *Aco4*, *Idh1, W1* loci were observed.

Introduction

Several diaphorase [EC.1.6.4.3] variabilities have been reported in soybean by using different electrophoretic methods (Gorman et al., 1983; Doong and Kiang, 1987; Rennie et al., 1989). The zymogram that we observed (Fig. 1) from our starch gel electrophoresis was similar to that reported by Rennie et al. (1989).

Sixteen different homozygous types of diaphorase were found in *G. max* and *G. soja* (Gorman, 1983). Each zymogram type was delineated by four distinct electrophoretic variants which were possibly controlled by variant alleles at four different loci (Gorman et al., 1983). Three *Dia* loci have been characterized in soybean. In the *Dia1* locus, the lowest five bands were controlled by the codominant alleles *Dia1-a* and *Dia1-b*. These five bands were believed to be mitochondrial-associated (Kiang and Gorman, 1983). Two codominant alleles, *Dia2-a* and *Dia2-b* were conditioned at the *Dia2* locus, which was believed to be cytoplasmic-associated (Gorman et al., 1983). The dominant allele, *Dia3-a*, conditioning the third fastest migrating cytoplasmic-associated band, and the recessive allele, *dia3-n*, conditioning the lack of this band, were at the *Dia3* locus (Kiang and Gorman, 1983). This paper reports a new diaphorase pattern with a null allele at the *Dia2* locus and investigates its inheritance and linkage relationships with other loci.

Materials and Methods

The parents used in this genetic study were PI 567565 (Shangdong, China) and the cultivar BSR 101 (Tachibana et al., 1987). Ten F1 seeds of this cross, BSR 101 x PI 567565, were produced at the Bruner Farm near Ames, IA. The F1 seeds were sent to Puerto Rico to produce F2 seeds, which were used for inheritance and linkage tests.

The parents and F_2 seeds were germinated in a dark growth chamber at 37°C for 48 hrs and a small piece of cotyledon was removed. The F_2 seedlings were returned to a lighted growth chamber for several days. The seedlings were transplanted to the greenhouse sandbench. After three weeks, hypocotyl color of each seedling was recorded as either purple (*W1/_*)or white (*w1/w1*).

The starch gel electrophoretic assay was adapted from Rennie et al. (1989) and Wendel and Weeden (1989). A 12.3 % starch gel was optimum for the diaphorase assay. The CT buffer system (pH=6.5) was used to assay the isozymes: diaphorase (*Dia2*) and aconitase (*Aco4*, EC 4.2.1.3). The CT electrode buffer (33.6 g citrate acid monohydrate, 46 ml N-(3-aminopropyl)-morpholine, and 4 L DD H_2O, pH=6.5) was diluted at the ratio of 1 electrode buffer : 19 DD H_2O to make the CT gel buffer. Then 60.7 g starch and 500 ml CT gel buffer were used to pour one CT gel. The CT gel was run at 9.5 W for 5.5 hrs. The B buffer system (pH=5.7) was used to assay isocitric dehydrogenase (*Idh2,* EC.1.1.1.42). The B electrode buffer (40.4 L-histidine, 16.0 g citrate acid monohydrate, 4 L DD H_2O, pH=5.7) was diluted at the ratio of 1 electrode buffer : 6 DD H_2O to make the B gel buffer. Then 60.7 g starch and 500 ml B gel buffer were used to make one B gel. The B gel was run at 9.5 W for 5.5 hrs. Staining methods were adapted from Rennie et al. (1989). Data were collected after staining the gels, and the data were analyzed by using the Linkage-1 computer program (Suiter et al., 1983).

Results and Discussion

The new diaphorase pattern showed two missing bands on the starch gel when compared to BSR 101. The two missing bands are produced at the *Dia2* locus. In the cross, BSR 101 X PI 567565, the *Dia2* pattern of F2 seeds approximated a 3 normal : 1 null ratio. This indicated that *Dia2-n* was a single recessive allele. Therefore, we propose the gene symbol, *Dia2-n*, for the allele conditioning the two missing bands. No linkage relationships were found between the *Dia2--Aco4*, *Dia2--Idh1*, *Dia2--W1* loci pairs. Locus *Dia2* has been reported linked with

fluorescent esterase (*Fle)* (Yu and Kiang, 1993) and was assigned to linkage group 21 (Palmer and Hedges, 1993). Kiang and Gorman (1983) suggested that there were two or three mitochondrial-associated and three or four cytosol-associated loci responsible for producing the full DIA zymogram pattern in soybean. According to our zymogram, using starch gel electrophoresis (Fig.1), only three possible *Dia* (*Dia1*, *Dia2*, and *Dia3*) loci were observed.

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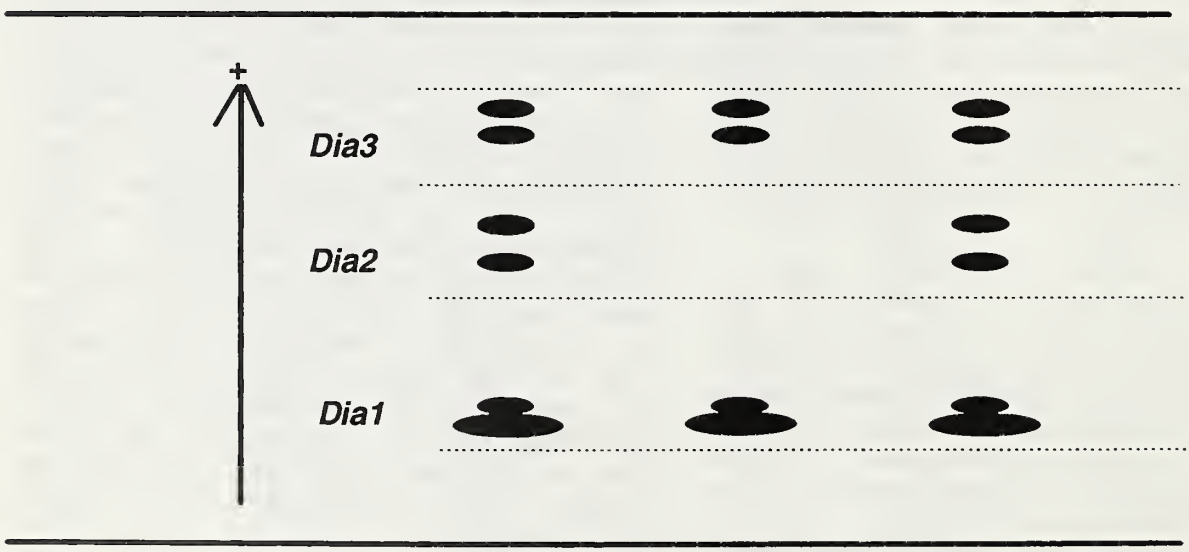

Locus	1	2	3
Dia3	*a/a*	*a/a*	*a/a*
Dia2	*a/a*	*n/n*	*a/a*
Dia1	*a/a*	*a/a*	*a/a*

Fig. 1 The DIA zymogram types of soybean

Table 1. Description of parental genotypes.

	Aco 4	*Idh1*	*Dia2*	Hypocotyl color
BSR 101	*a/a*	*a/a*	*a/a*	*W1/W1*
PI 567565	*b/b*	*b/b*	*n/n*	*w1/w1*

Table 2. Segregation in F2 seeds from the cross BSR 101 X PI 567565.

Locus	Alleles	Expected ratio	Observed ratio	N	X^2	P
Aco4	*a/a:a/b:b/b*	1:2:1	80:151:65	296	1.82	0.42
Idh1	*a/_ :b/b*	3:1	228: 72	300	0.48	0.49
Dia2	*a/_ :n/n*	3:1	222:76	298	0.12	0.74
Hypocotyl color	*W1/_: w1/w1*	3:1	188:73	261	3.68	0.06

Table 3. Segregation data of the *Dia2* locus with the *Aco4, Idh1,* and *W1* loci from the cross BSR 101 X PI 567565. (F2 data)

Locus pairs	Classes						N	X^2*	df	P †	‡R±SE
Dia2--	*a/_*	*a/_*	*a/_*	*n/n*	*n/n*	*n/n*					
Aco4	*a/a*	*a/b*	*b/b*	*a/a*	*a/b*	*b/b*					
	60	111	47	18	28	18	296	0.40	2	0.82	0.48±0.04
Dia2--	*a/_*	*a/_*	*n/n*	*n/n*							
Idh1	*a/_*	*b/b*	*a/_*	*b/b*							
	171	56	51	20			298	0.35	1	0.55	0.47±0.04
Dia2--	*a/_*	*a/_*	*n/n*	*n/n*							
W1	*W1/_*	*w1/w1*	*W1/_*	*w1/w1*							
	141	53	45	20			261	0.28	1	0.59	0.48±0.05

* Chi-square tests of independence were calculated by assuming either a 3:6:3:1:2:1 ratio in the F2 with 2 df or a 9:3:3:1 ratio in the F2 with 1 df.

† Probability of a greater value of chi-square.

‡ Recombination ± standard error.

Table 4. Linkage relationships of other loci from the cross BSR 101 X PI 567565. (F2 data)

Locus 1-- Locus 2	N	X^2*	df	P †	‡R±SE
Aco4--Idh1	296	0.071	2	0.96	0.49±0.04
Aco4--W1	261	1.34	2	0.51	0.48±0.04
Idh1--W1	261	0.27	1	0.60	0.48±0.04

* Chi-square tests of independence were calculated by assuming either a 3:6:3:1:2:1 ratio in the F2 with 2 df or a 9:3:3:1 ratio in the F2 with 1 df.

†Probability of a greater value of chi-square.

‡ Recombination ± standard error.

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Inheritance and Linkage Studies of an Aconitase 1 null in Soybean

Abstract

A new variant, aconitase 1 null (*Aco1-null*), was determined by electrophoretic technique in soybean [*Glycine max* (L.) Merr.]. Two *Aco1* variants, *Aco1-a* and *Aco1-b*, have been determined previously in soybean. Three different cross combinations involving the *Aco1-null* variant were made for inheritance and linkage tests. Inheritance tests showed that the *Aco1-null* was an allele at the *Aco1* locus and was recessive to *Aco1-a*. From the linkage analysis, no linkage relationships were observed between the *Aco1* locus and the *Aco2* (aconitase), *Fr1* (root fluorescence), *Pb* (pubescence tip), and *Pgm1* (phosphoglucomutase) loci.

Introduction

Aconitase is an important enzyme in the tricarboxylic acid cycle where it converts citrate to isocitrate. Aconitase is easy to detect on starch gel and five distinct major bands of aconitase have been observed (loci *Aco1, Aco2, Aco3, Aco4,* and *Aco5*). Mobility variants of five aconitase isozymes are conditioned by independent loci with codominant alleles [*Aco1(a, b), Aco2(a, b), Aco3(a, b), Aco4(a, b, c), and Aco5(a, b)*] (Amberger et al., 1992; Doong and Kiang, 1987; Griffin and Palmer, 1987; Palmer and Shoemaker, 1996).

The aconitase patterns of soybean PI 567502 and PI 567674 were different from previous aconitase patterns (Fig. 1). The least mobile band was missing and it seemed to be a null allele at the *Aco1* locus. The inheritance and linkage studies of *Aco1* have been reported. *Aco1* was conditioned by a single locus which displayed codominant expression of allele *a* and allele *b*. *Aco1* was not linked to any of the 11 loci tested (Griffin and Palmer, 1987). Thus, *Aco1* has not been assigned to any linkage group (Palmer and Hedges, 1993).

A mutation of *Aco2-b* to a null allele was reported in a somaclonal variant derived from the cultivar BSR 101 (Amberger et al., 1992). The linkage between *Aco1* and *Aco2* was not reported because the band produced by the *Aco1-b* allele migrated to a position nearly identical to that of the band produced by the *Aco2-a* allele, making it difficult to score F2 individuals accurately in a segregating population.

The purpose of this research was to investigate the inheritance of the aconitase 1 null pattern and to ascertain the linkage relationships between the aconitase 1 null and other loci.

Materials and Methods

The parents used in this genetic study were PI 567502, PI 567674, BSR 101, and Minsoy (PI 27890). PI 567502 is from Hebei, China, PI 567674 is from Henan, China, and BSR 101 is a commercial cultivar (Tachibana et al.,1987). Three crosses, BSR 101 x PI 567502, Minsoy x PI 567502, and BSR 101 x PI 567674 were made at the Bruner farm near Ames, IA. The F1 seeds were send to Puerto Rico to produce the F2 seeds which were used for inheritance and linkage tests.

The starch gel electrophoresis assay was adapted from Rennie et al. (1989) and Wendel and Weeden (1989). The parents and F2 seeds were germinated in a dark growth chamber at 37°C for 48 hrs and a small piece of cotyledon was removed. The F2 seedlings were returned to a lighted growth chamber for several days. Root fluorescence was observed under ultraviolet light. The seedlings were transplanted to the greenhouse sandbench. After two weeks, the pubescence tip of each seedling leaf was checked under the microscope. Two different kinds of pubescence tip (sharp pubescence tip, *Pb* and blunt pubescence tip, *pb*) were observed (Ting, 1946).

Eleven percent starch gel was optimum for the aconitase assay. The CT buffer system was used to assay the isozymes: aconitase (A*co1*, *Aco2*, and *Aco4*, EC 4.2.1.3) and phosphoglucomutase (*Pgm1*, EC 2.7.5.1). The CT electrode buffer (33.6 g citrate acid monohydrate, 46ml N-(3-aminopropyl)-morpholine, and 4 L DD H_2O, pH=6.5) was diluted at the ratio of 1 electrode buffer : 19 DD H_2O to make the CT gel buffer. And 55 g starch and 500 ml CT gel buffer were used to pour one CT gel. The CT gel was run at 9.5 Watts for 5.5 hrs. Data were collected after staining the CT gel.

The data were analyzed by using the Linkage-1 computer program (Suiter et al., 1983).

Results

Inheritance Test

Aconitase had five-band zones displayed on starch gel electrophoresis. *Aco1* was the least mobile zone among these five-band zones. *Aco1-null* displayed no color band in the *Aco1* band zone (Fig. 1). *Aco1-a* showed one band in the *Aco1* band zone. The F2 seeds of the three crosses involving *Aco1-null* and *Aco1-a* were tested. Chi-square tests of goodness of fit to a 3:1 ratio (*Aco1-a : Aco1-null*) were nonsignificant in these crosses (Table 2). This indicated that *Aco1-null* and *Aco1-a* were conditioned by a single locus with the dominant allele, *Aco1-a*, and the recessive allele, *Aco1-null*.

Linkage Determination

The F2 segregation data for dihybrids were analyzed for linkage relationships (Table 3). They were tested against five loci that gave 1:2:1 or 3:1 ratios in F2 The test showed that the *Aco1--Aco2, Aco2--Pb, Aco2--Pgm1, Aco4--Pgm1* loci fit a 1:2:1 x 3:1 ratio and that the *Aco1--Pb, Aco1--Fr1*, and *Aco1--Pgm1* loci fit a 3:1 x 3:1 ratio (Tables 4 and 5).

Discussion and Conclusion

The F2 segregation data of the three crosses, BSR 101 x PI 567502, Minsoy x PI 567502, and BSR 101 x PI 567674 indicated that the *Aco1-null* allele behaved as a recessive allele in the crosses with the *Aco1-a* allele. The *Aco1-a* allele was codominant with the *Aco1-b* allele at the *Aco1* locus (Amberger et al., 1987; Griffin and Palmer, 1987), therefore, we assumed that *Aco1-null* is recessive to *Aco1-b*.

The maximum-likelihood estimates of frequency of recombination showed that *Aco1* was independent of all the tested loci. The results indicated that *Aco1* had no linkage relationships with the other four loci, *Aco2*, *Pb*, *Fr1*, and *Pgm1*. Griffin and Palmer (1987) showed no linkage between *Aco1* and *Aco3*, *Aco4*, *Enp*, *Ap*, *Dia1*, *Ep*, *Idh1*, *Idh2*, *Sp1*, *T*, and *W1*.

The new variant, *Aco1-null* was identified in soybean, PI 567502 and PI 567674 . The variant was characterized by a missing band in the *Aco1* zone.

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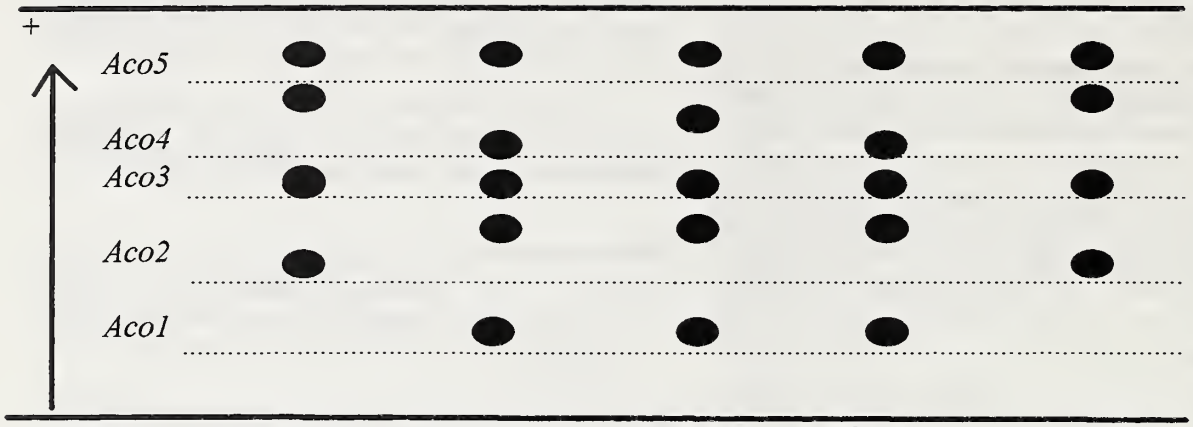

Locus	PI 567502	BSR 101	Minsoy	BSR 101	PI 567674
Aco4	*b/b*	*a/a*	*c/c*	*a/a*	*b/b*
Aco3	*a/a*	*a/a*	*a/a*	*a/a*	*a/a*
Aco2	*a/a*	*b/b*	*b/b*	*b/b*	*a/a*
Aco1	*null/null*	*a/a*	*a/a*	*a/a*	*null/null*

Fig. 1 The ACO zymogram of soybean

Table 1: Parental genotypes.

	Aco1	*Aco2*	*Aco4*	*Pgm1*	*Fr1*	Pubescence tip
PI 567502	*null*	*a/a*	*b/b*	*b/b*	*Fr1/Fr1*	*Pb/Pb*
PI 567674	*null*	*a/a*	*b/b*	*a/a*	*Fr1/Fr1*	*pb/pb*
BSR 101	*a/a*	*b/b*	*a/a*	*b/b*	*Fr1/Fr1*	*pb/pb*
Minsoy	*a/a*	*b/b*	*c/c*	*b/b*	*fr1/fr1*	*Pb/Pb*

Table 2: Inheritance of the *Aco1 null* (F2 data).

Cross	*a/-*	*null*	N	X^2(3:1)	P
BSR 101 X PI 567502	233	67	300	1.13	0.29
Minsoy X PI 567502	176	63	239	0.24	0.65
BSR 101 X PI 567674	215	72	287	0.001	0.99
Total	624	202	826	0.013	0.91

Table 3: F2 segregation data of the five loci .

Locus	Genotype	Expected ratio	Observed ratio	N	$X^2_{(1:2:1)}$ or $X^2_{(3:1)}$	P
Aco2[a]	*a/-:b/b*	3:1	216:84	300	1.44	0.24
Aco2[b]	*a/-:b/b*	3:1	187:54	239	0.74	0.41
Aco2[c]	*a/a:a/b:b/b*	1:2:1	68:128:73	269	0.81	0.67
Aco4[a]	*a/a:a/b:b/b*	1:2:1	76:164:60	300	4.32	0.12
Aco4[b]	*a/a:a/c:c/c*	1:2:1	59:119:61	239	0.038	0.98
Aco4[c]	*a/-:b/b*	3:1	224:63	287	1.42	0.24
Fr1[b]	*Fr1/-: fr1/fr1*	3:1	183:49	232	1.86	0.18
Pb[a]	*Pb/-: pb/pb*	3:1	147:55	202	0.53	0.47
Pgm1[c]	*a/-:b/b*	3:1	221:66	287	0.61	0.45

[a] BSR 101 X PI 567502

[b] Minsoy X PI 567502

[c] BSR 101 X PI 567674

Table 4: Linkage relationships of *Aco1-null* with other loci from three different cross combinations (F2 data).

Locus	N	X^2 †	df	P ‡
Aco2[a]	265	0.03	1	0.86
Aco2[b]	239	4.26	2	0.12
Aco2[c]	287	7.07	2	0.028
Fr1[b]	239	4.24	1	0.039
Pb[a]	193	1.19	1	0.27
Pgm1[c]	287	0.03	1	0.86

[a] BSR 101 X PI 567502.

[b] Minsoy X PI 567502.

[c] BSR 101 X PI 567674.

† Chi-square tests of independence were calculated by assuming either a 3:6:3:1:2:1 ratio in the F2 with 2 df or a 9:3:3:1 ratio in the F2 with 1 df.

‡ Probability of a greater value of chi-square.

Table 5: Linkage analysis of other loci in the three *Aco1-null* crosses (F_2 data)

Locus1-Locus2	N	X^2 †	df	P‡
Aco2-Pb[a]	193	0.02	1	0.89
Aco2- Pgm1[c]	287	3.31	2	0.19
Aco4- Pgm1[c]	287	0.73	1	0.40

[a] BSR 101 X PI 567502.

[b] Minsoy X PI 567502.

[c] BSR 101 X PI 567674.

† Chi-square tests of independence were calculated by assuming either a 3:6:3:1:2:1 ratio in the F2 with 2 df or a 9:3:3:1 ratio in the F2 with 1 df.

‡ Probability of a greater value of chi-square.

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Inheritance and Linkage Studies of 6-Phosphogluconate Dehydrogenase in Soybean

Abstract

A new variant, phosphogluconate dehydrogenase 1 null (*Pgd1-n*), was ascertained in Central Chinese soybean germplasm. Three alleles, *Pgd1-a, Pgd1-b*, and *Pgd1-c* at the *Pgd1* locus have been determined previously in soybean. The F2 seeds of the cross *Pgd1-b/Pgd1-b* (BSR 101) x *Pgd1-n/Pgd1-n* (PI 567399) were assayed. Inheritance tests showed that the *Pgd1-n* was recessive to *Pgd1-b* at the *Pgd1* locus. From the linkage analysis, the *Pgd1* locus was not linked with *Aco4* (aconitase), *W1* (hypocotyl color), *Pb* (pubescence tip), *Pgm1* (phosphoglucomutase), or *Idh2* (isocitrate dehydrogenase).

Introduction

The enzyme 6-phosphogluconate dehydrogenase [EC 1.1.1.44] is involved in the pentose phosphate pathway. It catalyzes the 6-phosphogluconate to ribulose 5-phosphate reaction and transfers H^+ to $NADP^+$ to produce NADPH.

6-phosphogluconate dehydrogenase was determined to be a dimeric isozyme by electrophoretic studies in several plant species (Gottlieb, 1981). In those plant species studied, 6-PGD isozymes usually were encoded by two loci, with products of one locus active in the cytoplasm and the products of the other locus associated with the plastids (Gottlieb, 1981).

Eight homozygous 6-PGD zymogram types with as many as six bands were found in soybean [*Glycine max* (L.) Merr.] and (*Glycine soja* Sieb. & Zucc.) (Gorman, 1983). In a genetic study in soybean, three 6-PGD loci were identified; *Pgd1*, *Pgd2*, and *Pgd3*. The *Pgd1* and *Pgd2* were two interacting loci and four bands were observed by starch gel electrophoresis. Band 1 (the least mobile band) was conditioned by the *Pgd1* locus with the *Pgd1-a*, *Pgd1-b*, and *Pgd1-c* alleles. Band 2 was an interlocus heterodimer of alleles at the *Pgd1* and *Pgd2* loci (Kiang and Gorman, 1983). Band 3 was controlled by the codominant alleles *Pgd2-a*, *Pgd2-b*, and *Pgd2-c* at the *Pgd2* locus (Chiang and Kiang, 1987). Band 4 was conditioned by the *Pgd3-a* and *Pgd3-b* alleles at the *Pgd3* locus (Chiang and Kiang, 1987). The *Pgd1* locus was linked with the *Pgi1* (phosphoglucose isomerase), *L1* (pod color), and *Dt1* (determinate stem) loci in linkage group 5 (Palmer and Hedges, 1993).

In a recent study of Central Chinese germplasm, soybean, PI 567399 was identified as having a different PGD pattern from the previous ones. The least mobile band was missing. This variant will be a good isozyme marker in linkage studies.

The F2 seeds from the cross BSR 101 X PI 567399 were assayed for inheritance and linkage relationships of this new variant with other loci.

Materials and Methods

The F2 seeds from the cross of BSR 101 (Tachibana et al., 1987) and PI 567399 (Shaanxi, China) were germinated in a dark growth chamber for 48 hrs. Sample preparation, electrophoretic techniques, and staining methods were adapted from Rennie et al. (1989) and Wendel and Weeden (1984). The CT gel system was applied for 6-phosphogluconate dehydrogenase [EC 1.1.1.44], aconitase [EC 4.2.1.3], and phosphoglucomutase [EC 2.7.5.1]. The B gel system was applied for the isocitrate dehydrogenase [EC 1.1.1.42] assay. Both the CT and B gel systems were run at 9.5 Watts for 5.5 hrs. Data were collected after staining and were analyzed by using the Linkage-1 computer program (Suiter et al., 1983).

Results and Discussion

Isozymes of 6-PGD normally have four bands displayed on starch gel electrophoresis. A new variant of 6-PGD in soybean PI 567399 was characterized by a missing band corresponding to the least mobile band (Fig. 1). This band was conditioned by the *Pgd1* locus with three codominant alleles. We proposed that this least mobile band was controlled by the *Pgd1-n* allele. The parental genotypes are given in Table 1. The F2 segregation data from the cross, BSR 101 (*Pgd1-b/Pgd1-b*) x PI 567399 (*Pgd1-n/Pgd1-n*), showed a 3 normal : 1 null ratio model (Table 2). This indicated single-gene inheritance of *Pgd1-n* with *Pgd1-n* as a recessive allele to *Pgd1-b*. Since *Pgd1-a, Pgd1-b* and *Pgd1-c*

were three codominant alleles, it was hypothesized that the *Pgd1-n* allele also was recessive to *Pgd1-a* and *Pgd1-c*. The results showed that *Pgd1* was not linked to any of the *Aco4*, *Idh2*, *Pgm1*, *W1*, or *Pb* loci (Table 3). Also no linkage relationships were detected between *Idh2--Pgm1, Idh2--W1, Idh2--Pb, Pgm1--Pb, and Pgm1--W1* (Table 4). Another 6-PGD null pattern was found in Central Chinese germplasm. Allelism test will be done to test these two mutants. A paper concerning this new variant will be reported later.

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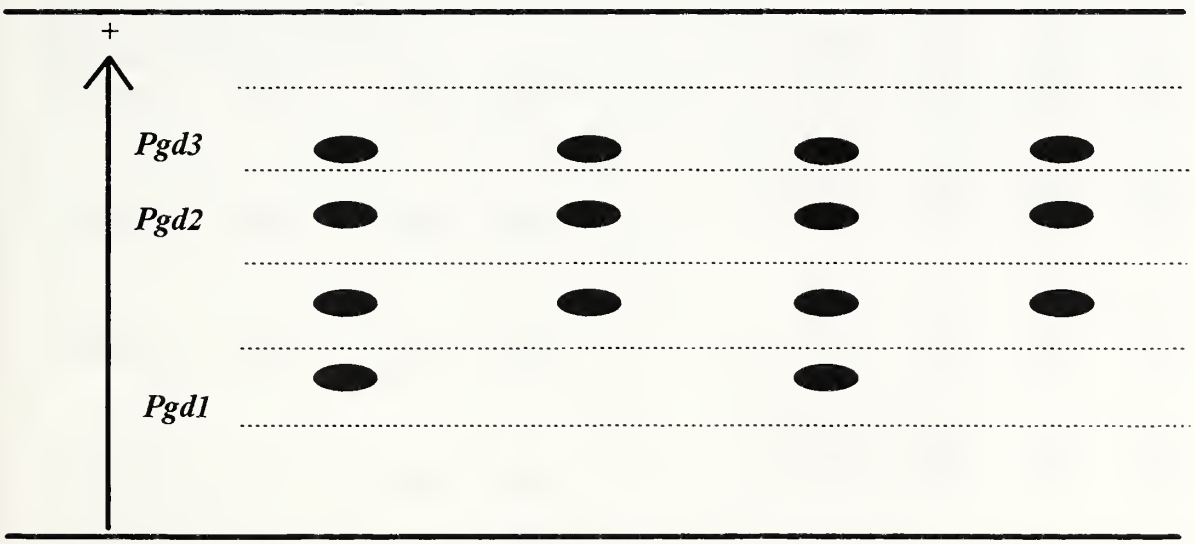

Locus	BSR 101	PI 567399	BSR 101	PI 567399
Pgd3	*b/b*	*b/b*	*b/b*	*b/b*
Pgd2	*a/a*	*a/a*	*a/a*	*a/a*
Pgd1	*b/b*	*n/n*	*b/b*	*n/n*

Fig. 1 The 6-PGD zymograms of soybean.

Table 1. Parental genotype description.

	Pgd1	*Aco4*	*Pgm1*	*Idh2*	*Pb*	*W1*
BSR 101	*b/b*	*a/a*	*b/b*	*b/b*	*pb/pb*	*W1/W1*
PI 567399	*n/n*	*b/b*	*a/a*	*a/a*	*Pb/Pb*	*w1/w1*

Table 2. Segregation analysis in the F2 population from the cross BSR 101 X PI 567399.

Locus	Alleles	Expected ratio	Observed ratio	N	X^2	df	P
Aco4	*a/a:a/b:b/b*	1:2:1	80:141:79	300	1.086	2	0.30
Idh2	*b/b:a/a*	3:1	223:76	300	0.03	1	0.88
Pgd1	*b/_: n/n*	3:1	237:63	300	2.56	1	0.11
Pgm1	*b/b:a/a*	3:1	217:83	300	1.14	1	0.29
Pb	*Pb/_:pb/pb*	3:1	201:78	279	1.30	1	0.26
W1	*W1/_:w1/w1*	3:1	204:75	279	0.53	1	0.48

Table 3. Linkage relationships of the *Pgd1* locus with the *Aco4, Idh2, Pb, Pgm1,* and *W1* loci from the cross BSR 101 X PI 567399. (F2 data)

Locus	Class						N	†X^2	df	‡P	§R±SE
Pgd1--	*b/_*	*b/_*	*b/_*	*n/n*	*n/n*	*n/n*					
Aco4	*a/a*	*a/b*	*b/b*	*a/a*	*a/b*	*b/b*					
	60	119	58	20	22	21	300	4.73	2	0.09	0.49±0.04
Pgd1--	*b/_*	*b/_*	*n/n*	*n/n*							
Idh2	*b/_*	*a/a*	*b/_*	*a/a*							
	177	59	46	17			299	0.10	1	0.75	0.49±0.04
Pgd1--	*b/_*	*b/_*	*n/n*	*n/n*							
Pb	*Pb/_*	*pb/pb*	*Pb/_*	*pb/pb*							
	164	54	37	24			279	5.02	1	0.02	0.41±0.05
Pgd1--	*b/_*	*b/_*	*n/n*	*n/n*							
Pgm1	*b/_*	*a/a*	*b/_*	*a/a*							
	173	64	44	19			300	0.25	1	0.62	0.48±0.04
Pgd1--	*b/_*	*b/_*	*n/n*	*n/n*							
W1	*W1/_*	*w1/w1*	*W1/_*	*w1/w1*							
	156	63	48	12			279	1.84	1	0.17	0.43±0.05

† Chi-square tests of independence were calculated by assuming either a 3:6:3:1:2:1 ratio in the F2 with 2 df or a 9:3:3:1 ratio in the F2 with 1 df.

‡ Probability of a greater value of chi-square.

§ Recombination ± standard error.

Table 4. Linkage relationships of other loci from the cross BSR 101 X PI 567399. (F2 data)

Locus	Class				N	†X^2	df	‡P	§R±SE
Idh2--Pgm1	*b/_ b/_*	*b/_ a/a*	*a/a b/_*	*a/a a/a*					
	157	66	59	17	299	1.48	1	0.22	0.45±0.05
Idh2--W1	*b/_ W1/_*	*b/_ w1/w1*	*a/a W1/_*	*a/a w1/w1*					
	152	58	51	17	278	0.18	1	0.67	0.48±0.05
Idh2--Pb	*b/_ Pb/_*	*b/_ pb/pb*	*a/a Pb/_*	*a/a pb/pb*					
	152	58	49	19	278	0.003	1	0.96	0.49±0.04
Pgm1--pb	*b/_ Pb/_*	*b/_ pb/pb*	*a/a Pb/_*	*a/a pb/pb*					
	142	57	59	21	279	0.27	1	0.60	0.48±0.05
Pgm1-W1	*b/_ W1/_*	*b/_ w1/w1*	*a/a W1/_*	*a/a w1/w1*					
	148	52	56	23	279	0.16	1	0.69	0.49±0.05

† Chi-square tests of independence were calculated by assuming either a 3:6:3:1:2:1 ratio in the F2 with 2 df or a 9:3:3:1 ratio in the F2 with 1 df.

‡ Probability of a greater value of chi-square.

§ Recombination ± standard error.

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Inheritance of Two Unknown EMS Generated Root Fluorescence Mutants

Introduction

Several root fluorescence mutants have been characterized in soybean, and used as genetic markers. The mutants squelch root fluorescence, appearing pink under ultraviolet light. Although root fluorescence mutants were discovered by analyzing spontaneous mutations, they also have been generated using several mutagens (Sawada and Palmer, 1987). The two mutations we chose to study were generated using EMS (ethyl methanesulfonate) treatment. The purpose of this experiment was to characterize the inheritance of these new mutations. This was accomplished by crossing the unknowns to several known root fluorescence mutants, and by making reciprocal crosses between the unknown root fluorescence mutants.

Materials and Methods

The unknown root fluorescence mutants were identified during a soybean mutagenesis treatment with EMS. Treatment was based upon earlier EMS protocol (Hammond and Fehr, 1984). These mutants were designated as EMS-19, and EMS-93. In order to characterize these mutants, crosses were made between the unknowns and previously identified root fluorescence mutants, *fr1*, *fr2*, *Fr3*, *fr4*, and *fr5*. The inheritance of these known mutants has been characterized in previous studies (Delannay and Palmer, 1982; Sawada and Palmer, 1987). The *Fr3* mutant is a dominant mutation. The *fr1*, *fr2*, *fr4*, and *fr5* mutants are located at separate loci, and are recessive. Reciprocal crosses also were made between EMS-19 and EMS-93 to test for allelism.

The F_1 plants were generated by crossing homozygous mutants at each of the mentioned *fr* or *Fr* loci with both unknown mutants. They were allowed to self pollinate, thus producing F_2 seed. The F_2 seed were labeled according to parentage and planted on germination paper. Root phenotype was visualized five days after germination with use of an ultraviolet light. Mutants were characterized as squelching root fluorescence under UV light, causing the root to appear pink. Wild type roots fluoresced, appearing white. The F_2 data were analyzed for significance by using the Chi-Square test.

Results

Two classes of F_2 progeny were identified (Table 1). The crosses between EMS-13 and *fr2*, and between EMS-93 and *fr2* resulted in all nonfluorescent F_2 families. The reciprocal crosses between EMS-13 and EMS-93 produced nonsegregating, non-fluorescent F_2 families. The crosses between the two unknowns (EMS-13 and EMS-93) and *fr1*, *fr4*, and *fr5* all resulted in segregating F_2 families. These F_2 progenies, 9 fluorescent : 7 nonfluorescent indicate nonallelism. Crosses between the two unknowns and *Fr3* conformed to the 3:13 ratio characteristic of a nonallelic relationship in which one loci is a dominant epistatic mutant.

Discussion

These results allow us to conclude that the two EMS induced mutants are allelic to each other and to the *fr2* mutant. Although these mutations may prove to differ on the molecular level, they present identical phenotypes when visualized with ultraviolet light. Crosses between the unknowns and the loci *fr1*, *Fr3*, *fr4* and *fr5* indicate nonallelism. Crosses between the unknowns and *Fr3* imply that *Fr3* is a dominant epistatic gene when in combination with recessive nonfluorescent mutants. These relationships are shown with levels of significance using chi-square analysis, and their associated P values.

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Table 1: Unknown homozygous root fluorescence mutants crossed to known homozygous root fluorescence mutants: F_2data

Segregating entries

Cross	No. F2 plants: Fluorescent	No. F2 plants: Nonfluorescent	$X^2_{(9:7)}$	P	$X^2_{(3:13)}$	P
fr$_1$ fr$_1$ x EMS-13	115	87	0.038	0.86	193.29	<<0.01
Fr$_3$ F$_3$ x EMS-13	49	172	104	<<0.01	1.69	0.19
fr$_4$ fr$_4$ x EMS-13	118	99	0.31	0.59	180	<<0.01
EMS-93 x *fr$_5$ fr$_5$*	120	90	0.07	0.82	203	<<0.01
fr$_1$ fr$_1$ x EMS-93	408	307	0.19	0.67	688.93	<<0.01
Fr$_3$ Fr$_3$ x EMS-93	49	169	101	<<0.01	1.98	0.13
fr$_4$ fr$_4$ x EMS-93	126	93	0.14	0.70	216	<<0.01
EMS-93 x *fr$_5$ fr$_5$*	100	87	0.58	0.46	148	<<0.01

Nonsegregating entries; No. F2 plants

Cross	Fluorescent	Nonfluorescent
EMS-13 x EMS-93	0	159
EMS-93 x EMS-13	0	57
fr$_2$ fr$_2$ x EMS-13	0	211
fr$_2$ fr$_2$ x EMS-93	0	222

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Isolation of a heterozygous female semisterile factor and its linkage relationship with three morphological markers in soybean

Introduction

Belling (1914) described a heritable type of sterility in *Stizolium*, whose phenotype was about 50% pollen grain and embryo sac abortion. He termed this phenotype semisterility. The 50% pollen and ovule abortion is due to the meiotic segregation of a heterozygous chromosomal translocation which produced deficiency/duplication gametes. Heritable female sterility in angiosperms has been reported to be associated with a variety of factors including the lack of fertilization, failure of embryo sac to complete its development, etc. (Mogensen, 1982).

In soybean, four partial female-sterile mutants, PS-1, PS-2, PS-3, and PS-4, have been described (Palmer et al., 1989; Pereira, 1994). PS-1, PS-2, PS-3, and PS-4 were recovered from a gene-tagging experiment (Palmer et al., 1989). These four partial-sterile mutations do not affect pollen viability. PS-1 is a true breeding female partial-sterile mutant that is conditioned by a single recessive nuclear gene mutation, while PS-2, PS-3, and PS-4 are maintained as heterozygotes. Upon self-pollination of PS-2, PS-3, and PS-4, heterozygotes produce approximately 1 fertile : 1 partial-sterile plant. PS-2, PS-3, and PS-4 alleles do not transmit the partial sterile trait through the female when partial-sterile mutants are used as female parents in cross-pollinations. PS-1 is not allelic to PS-2, PS-3, and PS-4 (Pereira, 1994).

Soybean mutant, USDA isoline L67-3483, originally was recorded as a tan saddle seed coat mutant, which was found by L. F. Williams in 1955 in X-rayed cultivar 'Clark' at Columbia, Missouri. In 1993, a female semisterile factor was identified in L67-3483. The semisterility was evident in F1 plants derived from crosses using L67-3483 as parent. L67-3483 is completely fertile upon self-pollination, however, when it is used in cross-pollinations, all F1 plants are semisterile. The F1 pollen grains appear to be normal as judged by I_2 KI staining. However, about 50% ovule abortion is observed at maturity. Self-pollination of the F1 plants produce F2 plants in an approximately 1 fertile : 1 semisterile ratio. This phenomenon has been observed when L67-3483 is used both as female and male parent. Preliminary genetic data suggest that the semisterility of the F1 plants derived from cross-pollinations using L67-3483 is distinct from the partial-sterility of PS-1, which is conditioned by a single nuclear recessive allele, and from the partial-sterility of PS-2, PS-3, and PS-4, which is only transmitted through the male parent. Nonsignificant pollen abortion is evident in L67-3483 heterozygotes, suggesting that the semisterility is not due to a chromosomal translocation.

The objectives of this study were to : (1) determine the inheritance of this heterozygous female semisterile factor; (2) investigate whether the heterozygous female semisterility with L67-3483 is due to the pleiotropic effect of the *k2* (tan saddle seed coat) allele or linked to the *k2* locus; (3) determine the linkage relation of this heterozygous female semisterile factor with two morphological markers, pubescence color (*T1*) and hypocotyl color (*W1*).

Materials and Methods

The experimental material, Clark isoline L67-3483, was obtained from R. L. Nelson, USDA-ARS, Urbana, Illinois. The phenotypes of L67-3483 are tan saddle seed coat (*k2*), purple hypocotyl and purple flower (*W1*), and brown pubescence (*T1*). X1878, a soybean breeding line, was obtained from Asgrow Seed Company, Ames, Iowa. The phenotypes of X1878 are yellow seed coat (*K2*), green hypocotyl and white flower (*w1*), and gray pubescence (*t*).

Standard soybean crossing techniques were used to obtain cross-pollinations (Walker et al., 1979). Seed coat color, flower color, and pubescence color were used as morphological markers to verify the success of cross-pollinations. Crosses were made at the Bruner Farm near Ames, Iowa, in summer of 1994. F1 seeds were advanced to F2 seeds at the Iowa State University-University of Puerto Rico Soybean Nursery, at Isabela Substation, Isabela, Puerto Rico, in spring of 1995. The F2 seeds were planted at the Bruner Farm in summer of 1995. Segregations for fertility/semisterility, seed coat color, and pubescence color were recorded at maturity. One F2 family was harvested individually. Progeny tests of the F2:3 seeds were conducted in the sandbench in the USDA greenhouse at Iowa State University for the segregation of hypocotyl color

(purple vs. green). The segregation data were analyzed by using the computer program LINKAGE-1, version 90 (Suiter et al., 1983).

Results and Discussion

Segregation of fertility : semisterility was observed in F2 plants derived from the cross of X1878 x L67-3483. Chi-square tests indicated nonsignificance for the segregation of 1 completely fertile : 1 semisterile plant in all 11 F2 families (Tables 1, 2, and 3). Both X1878 and L67-3483 gave completely fertile plants upon self-pollination. It is suggested that the F2 semisterile plants were plants that contained the female semisterile factor in the heterozygous condition. Since L67-3483 was generated by X-ray radiation, it was hypothesized that the semisterility may be due to the pleiotropic effect of the *k2* allele or chromosomal rearrangements near the *k2* locus. If correct, a close linkage should be detected between the *k2* locus and the heterozygous female semisterile factor. The segregations of seed coat color and fertility/semisterility were checked in the F2 plants. Chi-square tests for the segregation of 3 yellow seed : 1 tan saddle seed and 1 fertile : 1 semisterile F2 plants indicated nonsignificance (Table 1). No close linkage was detected between the *k2* locus and the heterozygous female semisterile factor. The recombination fraction between the *k2* locus and the female semisterile factor was about 49.12 ± 3.79% based on the pooled data from five F2 families (Table 1). Thus, it is concluded that the *k2* locus and the heterozygous female semisterile factor segregate independently, suggesting that the semisterile phenotype of L67-3483 is not due to the pleiotropic effect of the *k2* allele or to chromosomal rearrangements near the *k2* locus.

Chi-square tests for the segregation of 3 brown pubescence : 1 gray pubescence plant and 1 fertile : 1 semisterile plant indicated independence based on 526 F2 plants from five F2 families (Table 2). Thus, no linkage was detected between the *T1* locus and the heterozygous female semisterile factor. The recombination fraction between the *W1* locus and the heterozygous female semisterile factor was 45.06 ± 5.25% based on F2:3 progenies from 180 F2 plants (Table 3). Thus, it is suggested that the *W1* locus and the heterozygous female semisterile factor segregate independently.

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Table 1. F2 segregation data for linkage determination between tan saddle seed coat color (*k2*) locus and the heterozygous female semisterile factor in L67-3483 from the cross of X1878 x L67 3483

Families	Semisterile plants		Fertile plants		$X^2_{(1:1)}$ [a]	$P_{(1:1)}$	$X^2_{(3:1)}$ [b]	$P_{(3:1)}$	$X^2_{(1:1)(3:1)}$ [c]	$P_{(1:1)(3:1)}$	R ± SE (%) [d]
	Yellow seed	Tan saddle seed	Yellow seed	Tan saddle seed							
1	31	9	28	11	0.01	0.93	0.00	1.00	0.34	0.56	44.38 ± 9.69
2	28	8	27	16	0.62	0.45	1.22	0.27	2.08	0.15	36.02 ± 9.44
3	60	17	79	19	2.52	0.12	1.83	0.18	0.19	0.66	45.82 ± 6.53
4	44	15	51	12	0.13	1.73	0.54	0.47	0.72	0.40	42.93 ± 7.78
5	25	7	27	9	0.24	0.64	0.37	0.56	0.09	0.76	46.95 ± 10.49
Pooled data	188	56	212	67	2.34	0.14	0.61	0.45	0.08	0.78	49.12 ± 3.79
Pooled X^2 (df=4)					3.52	0.48	3.96	0.42	3.33	0.50	
Homogeneity X^2 (df=3)					1.18	0.76	3.35	0.35	3.25	0.37	

a : X^2 test for the goodness of fit of 1 semisterile : 1 fertile plant.

b : X^2 test for the goodness of fit of 3 yellow seed : 1 tan saddle seed.

c : X^2 for the goodness of fit of the segregation of 1 fertile : 1 semisterile plant and 3 yellow seed : 1 tan saddle seed.

d : Recombination fraction ± standard error

Table 2. F2 segregation data for linkage determination between pubescence color (*T1*) locus and the heterozygous female semisterile factor in L67-3483 from the cross of X1878 x L67-3483.

Families	Semisterile plants		Fertile plants		$X^2_{(1:1)}$ [a]	$P_{(1:1)}$	$X^2_{(3:1)}$ [b]	$P_{(3:1)}$	$X^2_{(1:1)(3:1)}$ [c]	$P_{(1:1)(3:1)}$	R ± SE (%) [d]
	Brown	Gray	Brown	Gray							
1	29	11	31	8	0.01	0.92	0.04	0.84	0.53	0.47	42.86 ± 9.67
2	24	12	30	13	0.62	0.43	1.86	0.17	0.09	0.77	48.40 ± 9.74
3	57	20	70	30	3.00	0.08	1.00	0.32	0.35	0.56	45.57 ± 6.49
4	49	10	40	23	0.13	0.72	0.27	5.91	5.91	0.00	31.26 ± 7.39
5	24	27	27	10	2.23	0.14	13.64	0.04	0.04	0.85	47.90 ± 10.42
Pooled data	183	61	198	84	2.75	0.10	1.85	0.17	1.50	0.22	45.20 ± 3.76
Pooled X^2 (df=4)					5.99	0.20	16.81	0.00	6.92	0.14	
Homogeneity X^2 (df=3)					3.24	0.36	14.96	0.00	5.42	0.14	

a : X^2 test for the goodness of fit of 1 semisterile : 1 fertile plant.
b : X^2 test for the goodness of fit of 3 brown pubescence : 1 gray pubescence plant.
c : X^2 for the goodness of fit of the segregation of 1 fertile : 1 semisterile plant and 3 brown pubescence : 1 gray pubescence plant.
d : Recombination fraction ± standard error.

Table 3. F2:3 segregation data for linkage determination between hypocotyl color (*W1*) locus and the heterozygous female semisterile factor in L67-3483 from the cross of X1878 x L67-3483.

Semisterile plants			Fertile plants			$X^2_{(1:1)}$ [a]	$P_{(1:1)}$	$X^2_{(1:2:1)}$ [b]	$P_{(1:2:1)}$	$X^2_{(1:1)(1:2:1)}$ [c]	$P_{(1:1)(1:2:1)}$	R ± SE (%) [d]
Purple	Segregating	Green	Purple	Segregating	Green							
28	42	20	21	47	22	0.00	1.00	0.57	0.75	1.38	0.50	45.06 ± 5.25

a : X^2 test for the goodness of fit of 1 semisterile : 1 fertile plant.
b : X^2 test for the goodness of fit of 1 purple : 2 segregating : 1 green hypocotyl plant.
c : X^2 for the goodness of fit of the segregation of 1 fertile : 1 semisterile plant and 1 purple : 2 segregating : 1 green hypocotyl plant.
d : Recombination fraction ± standard error.

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Linkage Test of T277H with T323

Buss (1983) described T277 as a single recessive gene (*ms*$_5$) for male-sterility, female-fertility in soybean. Carter and Burton (1992) reported a tight linkage between *ms*$_5$ and green cotyledon.

T323 is *y*$_{20}$ *y*$_{20}$ *Mdh1-n Mdh1-n* and was derived from a gene-tagging study (Hedges and Palmer, 1992). The *y*$_{20}$ *Mdh1-n* loci have not been located to a linkage group.

Our objective was to test for linkage between the *ms*$_5$ and *y*$_{20}$ *Mdh1-n* loci.

Materials and Methods

Cross-pollinations were made between *ms*$_5$ *ms*$_5$ x *y*$_{20}$*y*$_{20}$ *Mdh1-nMdh1-n* plants in the USDA glasshouse at Ames, Iowa. The F_1 seed were advanced to the F_2 at the University of Puerto Rico - Iowa State University soybean nursery at Isabela, Puerto Rico.

The F_2 seed were germinated on paper, cotyledon samples were taken for malate dehydrogenase (E.C.1.1.1.37) (Cardy and Beversdorf, 1984), seedlings were transplanted to peat pots, and then transplanted to the field. The yellow plants (*y*$_{20}$ *y*$_{20}$) were very small, weak, and had low survival. The green plants were classified for fertility/sterility at maturity. The fertile green plants were single-plant threshed and evaluated in the F_2-generation as progeny rows.

Results and Discussion

The F2 segregation was 158 green plants to 45 yellow (*y*$_{20}$ *y*$_{20}$ *Mdh1-n Mdh1-n*) plants, a good fit to a 3:1 ratio, χ^2 (3:1) = 0.87. All 158 green plants were *Mdh1* ____; there was no recombination between the y$_{20}$ and *Mdh1-n* loci. Hedges and Palmer (1992) reported that *y*$_{20}$ *Mdh1-n* behaved as a single recessive gene and believed that the two phenotypes were the result of two separate but very tightly linked loci. At maturity there were 118 fertile plants to 40 sterile (*ms*$_5$ *ms*$_5$) plants, a good fit to a 3:1 ratio, x2 (3:1) = 0.01.

A total of 95 F_2 plants were threshed individually and evaluated as F_2-plant progeny rows. The expected x^2 is based on a 1:2:2:4 ratio and the calculated x^2 value was 1.40 (Table 1). Thus the *ms*$_5$ locus is not linked to the *y*$_{20}$ *Mdh1-n* loci.

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Table 1. F_3 segregation from self-pollination of fertile green foliage F_2 plants from the cross T277 x T323.

Phenotype	No. F_2 families
All green, all fertile	12
All green, seg. sterile	25
Seg. yellow, all fertile	20
Seg. yellow, seg. sterile	38

χ^2(1:2:2:4) = 1.40

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A Mutable Flower Phenotype from Asgrow XP2214

In summer of 1990 at the Asgrow Seed Co. nursery at Ames, Iowa, Teresa Harper noticed one plant that had white, and mutable flowers in the purity grow-out of experimental line, XP2214. The normal characteristics of this line are purple flowers, tawny pubescence, buff hilum color, and brown pod walls. The plant was identified at early flowering, tagged and hand threshed.

Our first thought was that the mutable-flowered plant in XP2214 was a seed contamination of A25AF, the source of the w_4-mutable line (T322) or a contaminant resulting from cross-pollination with A25AF. The progeny of the mutable-flowered plant in XP2214 were identical to sibling plants of XP2214 for all characteristics except flower color.

The pedigree of A25AF (the experimental line in which the w_4-mutable (w_4-*m*) line was identified) is given on page 83 of Weigelt et al. (1990). The coefficient of parentage is the probability that two lines carry the same allele at a given locus because of descent from a common ancestor (St. Martin, 1982). The pedigree of XP2214 is presented in Figure 1. The coefficient of parentage between these two lines is 0.10. St. Martin (1982) found that the mean coefficient of parentage among the Northern maturity group 00 to IV varieties released from 1976 to 1980 was 0.25.

Materials and Methods

Harosoy and Harosoy isolines w_1 and w_4 were obtained from Dr. R. L. Nelson, USDA-ARS, soybean germplasm curator at the University of Illinois, Urbana. Cross-pollinations were made between white-flowered plants descended from XP2214 and the Harosoy genotypes. The F_1 and the F_2 plants were grown at the Bruner Farm near Ames, Iowa.

Results

The F_1 plants resulting from cross-pollinations of white-flowered XP2214 plants with Harosoy and Harosoy-*w*, gave all purple-flowered F_1 plants (Table 1). In the cross with Harosoy$W_1W_1W_4W_4$, the F_2 generation gave a close fit to the expected 3 purple : 1 white (Table 2). In the cross with Harosoy-w_1, the F_2 generation gave a close fit to the expected 9 purple : 7 white (Table 2). The white flowered plants, w_1w_1, and w_4w_4 (near-white) sometimes are difficult to distinguish from each other (Groose and Palmer, 1991) and from the double recessive class. The F_1 plants and all F_2 plants from the crosses of white-flowered XP2214 plants with Harosoy-w_4 were white-flowered.

Discussion

The data in Tables 1 and 2 provide compelling evidence that allelism is at the w_4 locus not the w_1 locus, which is the same locus for the Asgrow mutable line A25AF (Groose and Palmer, 1990).

The experimental line XP2214 and its two parents are purple flowered lines, presumably $W_1W_1W_4W_4$ genotype. The single mutable flowered plant observed in 1990 was probably at the F_6 generation. No mutable flowers were observed in the preceding generations. The mutation from w_4 to w_4-*m* * most likely occurred in the F_4 or F_5 generation.

It is very unlikely that the single mutable flowered plant in line XP2214 was a seed contaminant of T322 (w_4-*m*). All phenotypic characteristics of the single mutable plant and its progeny, except for flower color, were identical to sibling XP2214 plants. We believe that we can rule out cross-pollination from T322 many generations prior to the 1990 observation. Isozyme analyses (data not shown) of the progeny of the mutable-flowered plant in line XP2214 are in complete agreement with the isozyme profiles of its immediate two parents.

A gene-tagging study with the w_4-*m* * line has given variants (data not shown) that are different from the variants generated from a gene-tagging study with w_4-*m* (Palmer et al. 1989).

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Table 1. Genetic analysis of white-flowered plants, from mutable flowers of Asgrow experimental line XP2214, crossed with Harosoy flower-color near-isogenic lines; F_1 data.

Parents		F_1 Plants	Flower Color
Female	Male*	No.	Phenotype
Harosoy $W_1W_1W_4W_4$	white flower	10	Purple
Harosoy $w_1w_1W_4W_4$	white flower	16	Purple
Harosoy $W_1W_1w_4w_4$	white flower	21	Purple

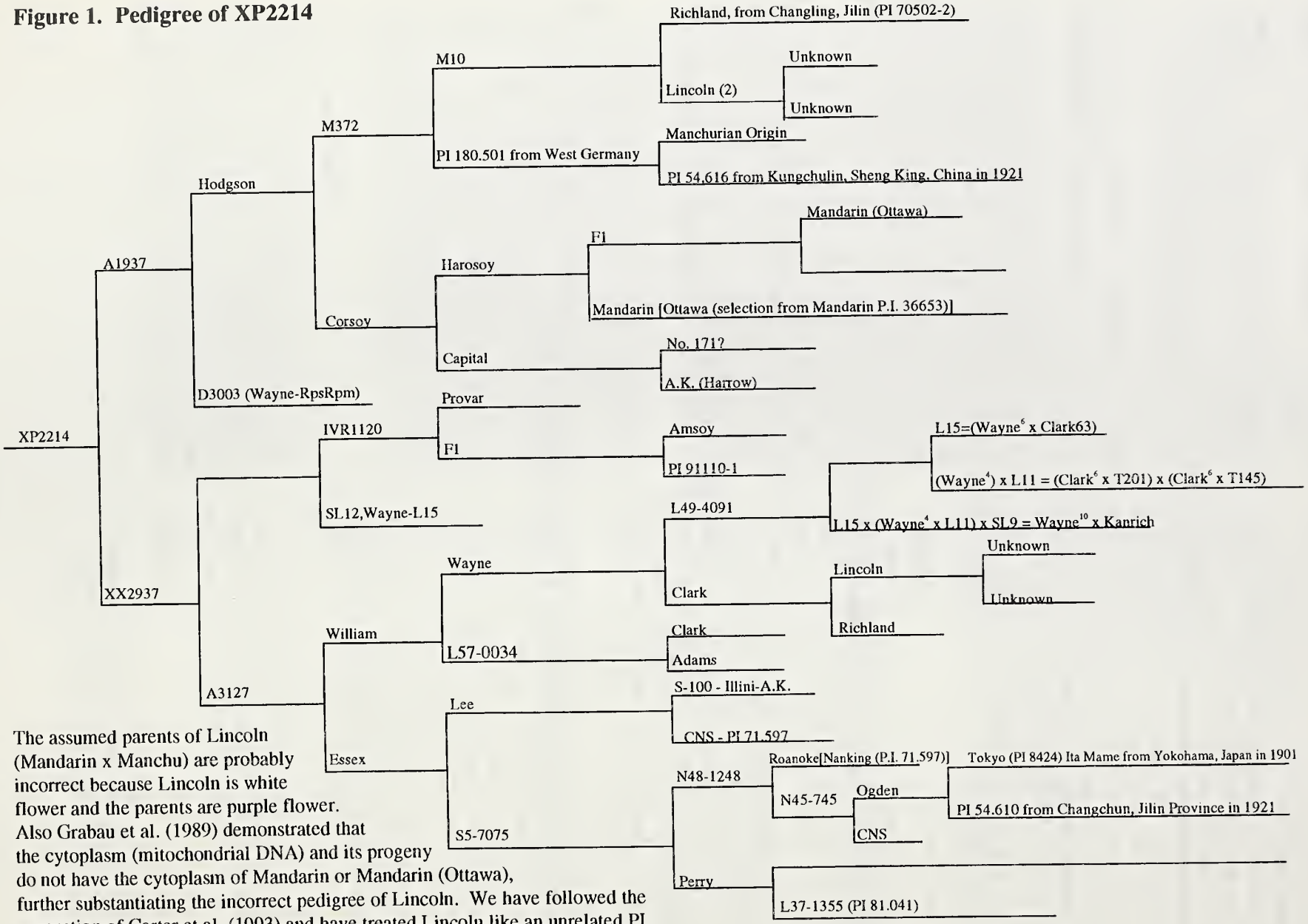
* White- flowered plants observed among the progeny of mutable-flowered plants of Asgrow line XP2214.

Table 2. Genetic analysis of white-flowered plants, from mutable flowers of Asgrow experimental line XP2214, crossed with Harosoy flower-color near-isogenic lines; F_2 data.

Parents		No. F_2 plants and flower color					
Female	Male	Purple	White*	$\chi^2_{(3:1)}$	P	$\chi^2_{(9:7)}$	P
Harosoy $W_1W_1W_4W_4$	white flower	803	279	0.36	0.56		
Harosoy $w_1w_1W_4W_4$	white flower	984	811			1.49	0.23
Harosoy $W_1W_1w_4w_4$	white flower	0	1887				

* A few mutable-flowered plants were observed and they were counted as white-flowered plants.

Figure 1. Pedigree of XP2214



The assumed parents of Lincoln (Mandarin x Manchu) are probably incorrect because Lincoln is white flower and the parents are purple flower. Also Grabau et al. (1989) demonstrated that the cytoplasm (mitochondrial DNA) and its progeny do not have the cytoplasm of Mandarin or Mandarin (Ottawa), further substantiating the incorrect pedigree of Lincoln. We have followed the suggestion of Carter et al. (1993) and have treated Lincoln like an unrelated PI.

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A new Variant of Phosphoglucose Isomerase

Abstract

A new phosphoglucose isomerase zymogram type with two extra bands was investigated in Central Chinese germplasm by starch gel electrophoresis. A 1 normal : 2 heterozygous: 1 new type ratio was obtained from the F2 seeds in the cross of Cutler 71 (normal type) and PI 567635 (new type). The two extra bands are suggested to be produced by a new locus, not by *Pgi1, Pgi2,* or *Pgi3.* This proposed new type has no linkage relationships with the *Aco3, Aco4, Ap, Pgd1, Pgm1*, and *W1* loci.

Introduction

Phosphoglucose isomerase (PGI) [EC 5.3.1.9] (sometimes called glucose phosphate isomerase, GPI, or phosphohexose isomerase, PHI) is one of the glycolytic enzymes studied in plant species. In most plant species, PGI are dimeric isozymes and usually are encoded by two loci. The product of one locus is active in the cytoplasm and the other is active in the plastids (Gottlieb, 1981).

Four homozygous PGI zymogram types have been reported in cultivated and wild soybean (Gorman et al., 1983). Four to nine bands may be seen for homozygous lines (Rennie et al., 1989). The zymogram types were hypothesized to be the product of three loci, *Pgi1, Pgi2*, and *Pgi3* (Kiang and Gorman, 1983). There were two codominant alleles *Pgi1-a* and *Pgi1-b* at the *Pgi1* locus (Rennie et al., 1989). A dominant allele, *Pgi2*, and a null allele, *pgi2*, were verified at the *Pgi2* locus (Chiang and Kiang, 1987). There are two codominant alleles *Pgi3-a* and *Pgi3-b* at the *Pgi3* locus (Chiang and Kiang, 1987). The *Pgi1* and *Pgi3* loci were hypothesized to produce a cluster of interlocus heterodimer bands (Gorman et al., 1983). The *Pgi1* locus is linked with the *Pgd1, Dt1*, and *L1* loci in linkage group 5 (Palmer and Hedges, 1993).

Chinese accession PI 567635 was characterized as having a new PGI pattern. Two extra bands (cluster of bands) were displayed on starch gel in comparison with the previous types (Fig. 1). A genetic analysis of this new pattern is reported in this paper.

Materials and Methods

PI 567635 was obtained from Dr. R. L. Nelson, USDA-ARS (Urbana, Illinois). The F2 seeds of the cross Cutler 71 and PI 567635 were germinated in a dark growth chamber for 2 days. Approximately 10 mg cotyledonary tissue was sampled for isozyme assay. Then the germinated seeds were transplanted to the greenhouse sandbench for scoring of hypocotyl color (*W1* locus). Isozymes 6-phosphogluconate dehydrogenase [EC 1.1.1.44], phosphoglucomutase [EC 2.7.5.1], and aconitase [EC 4.2.1.3] were assayed in the CT gel system. The B gel system was used for the isocitrate dehydrogenase [EC 1.1.1.42] and phosphoglucose isomerase [EC 5.3.1.9] assay. Stain recipes were adapted from Rennie et al. (1989). The Chi-square test was used to determine whether the *Pgi* and other loci assorted independently. The computer program Linkage-1 was used to calculate the recombination frequency (Suiter et al., 1983).

Results and Discussion

The PGI new pattern displayed two extra bands on starch gel electrophoresis. The parental genotypes are given in Table 1. The F2 segregation data showed a 1 normal : 2 heterozygous : 1 new type ratio (Table 2). A locus, in addition to *Pgi1, Pgi2,* and *Pgi3,* with codominant alleles was proposed to explain the genetic behavior of these bands. This proposed new locus was not linked to the *Aco3, Aco4, Ap, Pgm1, Pgd1, and W1* loci (Table 3). The two extra bands were produced by a new locus, not the *Pgi1* locus, since the *Pgi1* locus is linked with the *Pgd1* locus. No linkages were detected between *Aco3--Pgm1, Aco3--Pgd1, Aco3--W1, Ap--Pgm1, Ap--Pgd1,* and *Ap--W1* (Table 4).

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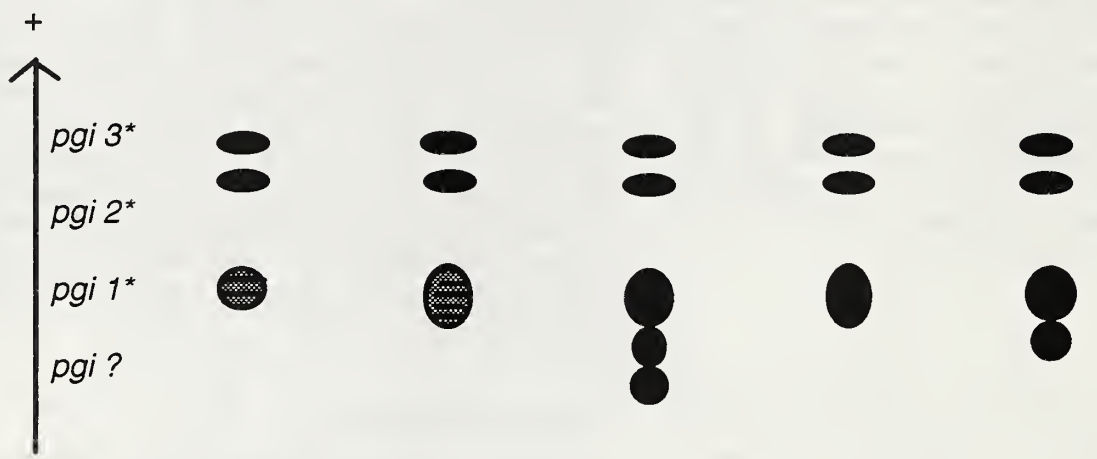

Locus	BSR 101	Cutler 71	PI 567635	Cutler 71	Heterozygous[a]
Pgi3	*b/b*	*b/b*	*b/b*	*b/b*	*b/b*
Pgi2	*pgi2/pgi 2*	*pgi2/pgi2*	*pgi2/pgi2*	*pgi2/pgi2*	*pgi2/pgi2*
Pgi1	*b/b*	*a/a*	*a/a*	*a/a*	*a/a*
Pgi?	*n/n*	*n/n*	*a/a*	*n/n*	*a/n*

[a] The pattern of an F2 seed.
* Pattern acccording to PGI starch gel zymograms from Rennie et al. (1989).

Fig. 1 The PGI zymogram of soybean

Table 1. Parental genotype description.

	Aco3	*Aco4*	*Ap*	*Pgi?*	*Pgd1*	*Pgm1*	*W1*
Cutler 71	*a/a*	*a/a*	*b/b*	*n/n*	*b/b*	*b/b*	*W1/W1*
PI 567635	*b/b*	*b/b*	*a/a*	*a/a*	*a/a*	*a/a*	*w1/w1*

Table 2. Segregation analysis in the F2 population from the cross of Cutler 71 X PI 567635.

Locus	Alleles	Expected ratio	Observed ratio	N	X^2	df	P
Aco3	*a/a:a/b:b/b*	1:2:1	81:133:85	299	3.91	2	0.15
Aco4	*b/_:a/a*	3:1	224:75	299	0.001	1	0.99
Ap	*b/b:b/a a/a*	1:2:1	68:162:69	299	2.10	2	0.36
Pgd1	*a/a/:a/b:b/b*	1:2:1	81:149:69	299	0.97	2	0.62
Pgm1	*b/b:a/a*	3:1	213:86	299	2.26	1	0.14
Pgi?	*a/a:a/n:n/n*	1:2:1	74:140:85	299	2.02	2	0.38
W1	*W1/_:w1/w1*	3:1	201:76	277	0.88	1	0.36

Table 3. Linkage relationships of the *Pgi?* locus with the *Aco3, Aco4, Ap, Pgm1, Pgd*1, and *W1* loci from the cross of Cutler 71 x PI 567635; F2 data.

Locus tested	N	†X^2	df	‡P	§R±SE
Aco3	299	3.14	4	0.53	0.49±0.03
Aco4	299	1.25	2	0.53	0.47±0.04
Ap	299	4.02	4	0.40	0.47±0.03
Pgm1	299	1.20	2	0.55	0.49±0.04
Pgd1	299	0.45	4	0.98	0.49±0.03
W1	277	0.04	2	0.98	0.49±0.04

† Chi-square tests of independence were calculated by assuming a 1:2:1:2:4:2:1:2:1 ratio in the F2 with 4 df, a 3:6:3:1:2:1 ratio in the F2 with 2 df, or a 9:3:3:1 ratio in the F2 with 1 df.

‡ Probability of a greater value of chi-square.

§ Recombination ± standard error.

Table 4. Linkage determination of other loci from the cross of Cutler 71 x PI 567635; F2 data.

Locus 1 -- Locus 2	N	†X^2	df	‡P	§R±SE
Aco3 -- Pgm1	299	0.24	2	0.89	0.48±0.03
Aco3 -- Pgd1	299	1.39	4	0.85	0.48±0.03
Aco3 -- W1	277	2.99	2	0.22	0.48±0.04
Ap -- Pgm1	299	7.38	2	0.02	0.49±0.04
Ap -- Pgd1	299	3.74	4	0.44	0.48±0.03
Ap -- W1	277	1.01	2	0.60	0.48±0.04

† Chi-square tests of independence were calculated by assuming a 1:2:1:2:4:2:1:2:1 ratio in the F2 with 4 df, a 3:6:3:1:2:1 ratio in the F2 with 2 df, or a 9:3:3:1 ratio in the F2 with 1 df.

‡ Probability of a greater value of chi-square.

§ Recombination ± standard error.

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Soybean Linkage Studies

Introduction

Mutations that cause lesions resembling those produced by pathogens have been reported in several plant species. Three necrotic root mutants were identified among the germinal revertant progeny of the w_4-*m* line in soybean (Palmer et al., 1989). When grown in the field, necrotic root plants do not survive, but in the glasshouse, they can be grown to maturity, especially if propagated as shoot scions on nonmutant root stocks. The three mutants each were inherited as a single recessive gene and were allelic (Kosslak et al., 1996).

Materials and Methods

Cross-pollinations were made between each of the three necrotic root mutants (T328H, T329H, and T330H) and T261 (in the cultivar 'Mandarin Ottawa' that has tan-saddle seed, k_2k_2, and is null for malate dehydrogenase, (*Mdh1-n Mdh1-n*).

Starch gel electrophoresis techniques described by Cardy and Beversdorf (1984a, b) were used to determine zymogram patterns. The B-gel system was used to assay for phosphoglucomutase (*Pgm1*, EC 2.7.5.1) and malate dehydrogenase (*Mdh1*, EC 1.1.1.37). The D-gel system was used to assay for aconitase (*Aco4*, EC 4.2.1.3), diaphorase (*Dia1*, EC 1.6.4.3), and isocitrate dehydrogenase (*Idh1*, EC 1.1.1.42).

For linkage determinations, F_2 phenotypic data were analyzed using the Linkage-1 computer program, which calculates linkage values by maximum likelihood (Suiter, et al., 1983).

Results and Discussion

No linkage was detected between the three necrotic root mutations and *Mdh1, Aco4, Dia1, Pgm1, and Idh1* (Kosslak et al., 1996). The other possible linkage combinations involved the five isozyme loci. No linkage was detected between any of the five isozyme loci (Table 1).

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Table 1. F_2 segregation data from crosses of the three necrotic root mutant soybean plants with T261. Total number of F_2 plants tested was 256 for each genetic combination.

Gene pair	df	x^{2d}	P
Aco4 - Dia 1[a]	8	7.36	0.50
Aco4 - Idh 1	8	8.67	0.37
Aco4 - Mdh 1	5	2.83	0.73
Aco4 - Pgm	8	4.41	0.82
Dia 1 - Idh 1	8	7.31	0.50
Dia 1 - Pgm	8	4.84	0.78
Dia 1 - Mdh 1	5	10.46	0.06
Idh 1 - Pgm	8	3.97	0.86
Idh 1 - Mdh 1	5	4.46	0.49
Mdh 1 - Pgm	5	8.18	0.15
Aco4 - Dia 1[b]	8	10.49	0.26
Aco4 - Idh 1	8	8.55	0.38
Aco4 - Mdh 1	5	2.43	0.79
Aco4 - Pgm	8	3.83	0.87
Dia 1 - Idh 1	8	4.57	0.80
Dia 1 - Pgm	8	12.61	0.13
Dia 1 - Mdh 1	5	4.71	0.45
Idh 1 - Pgm	8	8.97	0.34
Idh 1 - Mdh 1	5	4.33	0.50
Mdh 1 - Pgm	5	6.36	0.27
Aco4 - Dia 1[c]	8	9.48	0.30
Aco4 - Idh 1	8	6.73	0.57
Aco4 - Mdh 1	5	3.24	0.66
Aco4 - Pgm	8	10.43	0.24
Dia 1 - Idh 1	8	10.18	0.25
Dia 1 - Pgm	8	9.98	0.27
Dia 1 - Mdh 1	5	6.71	0.24
Idh 1 - Pgm	8	6.59	0.58
Idh 1 - Mdh 1	5	2.24	0.82
Mdh 1 - Pgm	5	3.14	0.68

[a] Cross: Heterozygous necrotic-root plants (T328H) x T261.

[b] Cross: Heterozygous necrotic-root plants (T329H) x T261.

[c] Cross: Heterozygous necrotic-root plants (T330H) x T261.

[d] Chi-square goodness of fit tested to a 1:2:1:2:4:2:1:2:1 or 6:3:3:1:2:1 depending upon the genetic segregation.

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Molecular Mapping of the *Shr* and *Cgy1* genes in Soybean

Abstract

The *Cgy1* and *Shr* genes control the α'-subunit of β-conglycinin and shriveled seeds, respectively. *Shr* was mapped on Linkage Group F in two independent populations. *Cgy1* also was mapped on Linkage Group F with a distance of 19.2 cM from the *Shr* gene. Both genes were integrated into USDA-ARS/ISU Soybean Linkage Map using the JoinMap program.

The *Cgy1* gene encodes the α'-subunit of β-conglycinin (Lelievre et al., 1992). A null allele in the *Cgy1* gene was found in the cultivar Keburi (Kitamura et al., 1984). Linkage analysis showed that the *Cgy1* gene segregates independently of the *Cgy2* and *Cgy3* genes which encode α- and β-subunits of β-conglycinin, respectively (Davies et al., 1985). The *Shr* gene conditions shriveled seeds. A mutant in *Shr* has been assigned Genetic Type Collection Number T311. The mutant T311 is controlled by a single recessive gene, with partial penetrance and incomplete expressivity (Honeycutt et al., 1989). Our objective was to map *Cgy1* and *Shr* on the USDA-ARS/ISU Soybean Linkage Map.

Two independent F2 populations were developed for mapping the *Shr* and *Cgy1* genes. Ninety-four F2 plants from the cross Keburi x T311 and 92 F2 plants from the cross Raiden x T311 were grown in growth chambers at 26/20°C (day/night) and a 15 h photoperiod for four weeks, then at 34/20°C and a 12 h photoperiod until maturity. Mature F2:3 seeds from each individual plant were harvested and stored in a cold room. Ten or more seeds from each F2:3 line were grown in a field near Ames, Iowa during the summer of 1996.

The phenotype of F2 plants grown in growth chambers was classified as shriveled or round based on the shriveled seed percentage with reference to the parental values. That is, a plant bearing more than 60% shriveled seeds was considered to be shriveled while a plant bearing less than 40% shriveled seeds was considered to be round. A total of 30 F4 seeds from each F3 plant was evaluated phenotypically to determine the genotype of the F2 progenitors. Each F3 plant was assessed as either shriveled or round based on the presence or absence of shriveled seeds among the 30 inspected F4 seeds. Lines in which scores disagreed between the phenotype determined with F2 plants in growth chambers and the genotypes determined with F3 plants in a field were eliminated, as were lines with five or fewer individuals. In the Raiden x T311 population, the genotypes of F2 plants segregated as 18 *ShrShr*: 30 *Shrshr*: 14 *shrshr*. In the Keburi x T311 population, F2 plants segregated as 16 *ShrShr*: 35 *Shrshr*: 14 *shrshr*. Segregation of *Shr* in both populations fit a single locus model with a segregation ratio of 1:2:1 (χ^2 = 0.58 and 0.51 for the Raiden x T311 population and the Keburi x T311 population, respectively).

The α'-subunit of β-conglycinin was detected with SDS-PAGE as described by Diers et al. (1994). The *Cgy1* genotypes of F2 plants were determined by scoring the presence or absence of the α'-subunit in each of six or more F2:3 seeds from each F2 plant. Segregation was 18 *Cgy1Cgy1*: 50 *Cgy1cgy1*: 25 *cgy1cgy1* in the Keburi x T311 population. The segregation of *Cgy1* fit a single locus model with the segregation ratio 1:2:1 (χ^2 =1.86).

For RFLP analysis, the DNA was extracted from the combined leaves harvested from seven or more F3 seedlings derived from each selfed F2 plant. DNA extraction, Southern blotting and hybridization were performed as described by Keim et al. (1989). Two hundred and forty eight probes covering all soybean linkage groups (USDA-ARS/ISU Soybean Linkage Map) at intervals of less than 20 cM initially were screened with five restriction enzymes, (*Hind*III, *Eco*RI, *Eco*RV, *Dra*I and *Taq*I) to detect polymorphisms between Raiden and T311 and between Keburi and T311. Among them, 87 probes for Raiden vs. T311 and 90 probes for Keburi vs. T311 were polymorphic with at least one of the five restriction enzymes. The Mapmaker program (Lander et al., 1987) was used to construct the linkage map by using the RFLP data and phenotype data of shriveled seeds and storage protein subunit. The LOD score of 3.0 was used as the lower limit for accepting linkage between two markers. After the genes were mapped, probes flanking the loci were assayed for polymorphism with additional restriction enzymes: *Hae*III, *Xba*I, *BamH*I, *Sph*I. The JoinMap program (Stam and Van Ooijen, 1995) was used to integrate these mapped markers and genes of both populations into USDA-ARS/ISU Soybean Linkage Map (Shoemaker et al., 1995).

The linkage analysis with the Raiden x T311 population showed that the *Shr* gene was linked to RFLP loci A_708D_1 and K_644D_1 on Linkage Group F with the LOD values of 12.6 and 6.0, respectively. Further analysis found that B_212T_1 and K_7H_2 were also linked to the *Shr* gene (Fig.1A). Linkage analysis with the Keburi x T311 population also indicated that the *Shr* gene was linked to A_708D_1 and B_212T_1 on Linkage Group F with the LOD values of 4.7 and 1.6, respectively (Fig. 1B). Markers B_148V_1, K_644D_1, A_186T_1, and A_806D_1 also were mapped to their corresponding positions (reference to USDA-ARS/ISU Soybean Linkage Map). Therefore, linkage analyses with two independent populations located the *Shr* gene on Linkage Group F. In the Keburi x T311 population, the *Cgy1* gene was linked to *Shr* at a distance of 19.2 cM and with a LOD value of 4.7 (Fig.1B). The mapped positions of *Shr* and *Cgy1* on USDA-ARS/ISU Soybean Linkage Map were shown in Fig. 1C.

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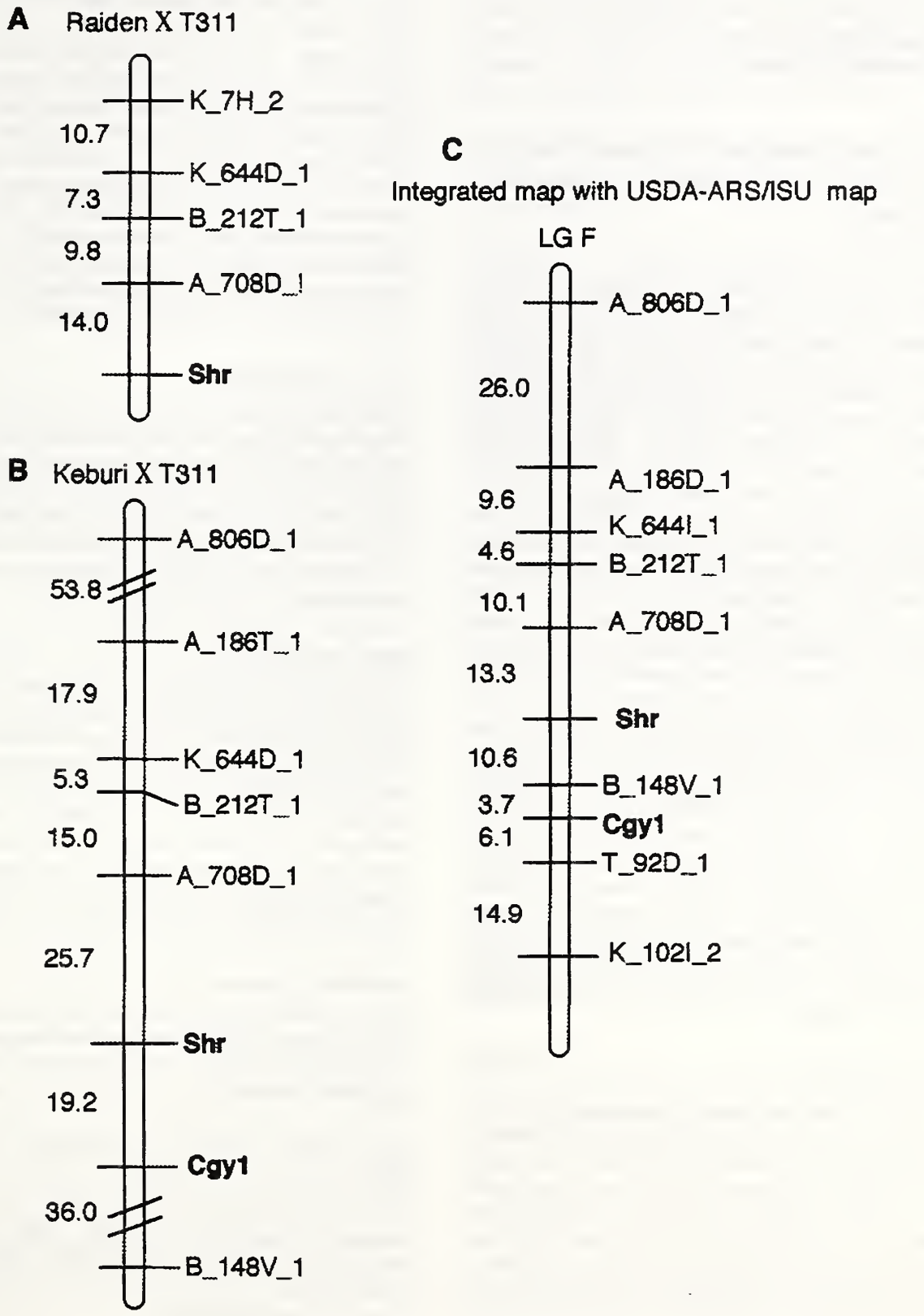
Figure 1. Summary of linkage in the Raiden x T311 population (**A**) and Keburi x T311 population (**B**) and integrated map with USDA-ARS/ISU Soybean Linkage Map (**C**). The distances in centiMorgan in **A** and **B** were computed by using the program Mapmaker (Lander et al., 1987). The mapped markers and genes in the both populations were integrated into USDA-ARS/ISU Soybean Linkage Map by using JoinMap program (Stam and Van Ooijen, 1995).



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NODULATION AND NITROGEN FIXATION IN SOYBASE, A SOYBEAN GENOME DATABASE

Prior to 1997, SoyBase, a soybean genome database, focused exclusively on genes, traits, loci, nodulins, enzymes, reactions, pathology, mapping information, QTL's, storage proteins, and germplasm data of soybean. The flexibility of the database allows a broad interpretation of data considered valuable to molecular biologists, geneticists and breeders. Nodulation and biological nitrogen fixation involve bacterial enzymes, signals, and metabolites, as well as those of the plant. Because of the importance of nodulation and dinitrogen fixation to soybean and to other legumes, these processes have now been incorporated into SoyBase. This represents the first comprehensive, up-to-date overview of these topics available to the public by computer, and it will be a valuable tool for this aspect of legume research.

Nodulation may be explored by "clicking" on that word in the list of classes, or subjects, in SoyBase. The broad categories of nodulation are 1) Nodule initiation; 2) Nodule development; 3) Nodule maturation; 4) Nodule senescence; 5) Plant gene symbols, map location, and linkage data; 6) Soybean nodulation mutants; 7) Source material, including cultivar and bacterium; and 8) Regulation. Each category may be further studied by following the desired hypertext links. Complete reference citations are included. A brief summary of the nodulation data in SoyBase follows.

Nodule initiation

Nodules are initiated through a combination of plant signals such as flavonoids and phenolics as well as rhizobial signals known as nod factors. Nod factors are lipochito-oligosaccharides and have been purified from several rhizobia, including *Bradyrhizobium japonicum*, the most common symbiont of soybean. There are references for nod factor structure in several legumes. Host specificity is determined by a wide variety of loci, including *nodABC*, *nodD*, *nolA*, *nodV*, and *nodW* . The developmental steps of nodule initiation are enumerated, from depolarization of the plasma membrane to curling of the root hair to formation of the infection tube and emergence of the nodule. Rhizobial nod genes (*Nod*, *Nol*, and *Noe*) are described by function and source. For example, *nodA* codes for an N-acetylglucosaminosyl N-acyltransferase involved in nod-factor synthesis and it is found in some form in all rhizobia. *NodD* is a transcriptional activator of other nod genes and is inducible in *B. japonicum*. Other rhizobial genes affect nodulation but may have other cellular functions as well. These include *ndv* involved in glucan synthesis, *hem* in heme synthesis, and *trp* in tryptophan synthesis. A large number of microbial mutants are referenced, because these have led to the elucidation of the genetics of nodulation.

Nodule development

Cytological changes include an increase of bacteroid tissue, enlargement of vacuoles, and formation of vascular bundles. Several early nodulins, notably Ngm ENOD40 and Ngm ENOD55 are important during this stage of development. All nodulins may be examined further by following hypertext links to the Nodulin class. Nodule development may be regulated enzymatically, by chitinase.

Nodule maturation

As maturation proceeds, the plant cell wall hydrolyses, and the nodule differentiates into an outer cortex region of loosely bound cells, an inner cortex which functions as an oxygen barrier, and a central core of large infected cells. The peribacteroid membrane forms from the plasma membrane. Peribacteroid nodulins and other late nodulins are enumerated. Enzymatic activity of the mature nodule is extensive and may be examined in detail by following hypertext links of more than 80 enzymes.

Nodule senescence

Rapid senescence starts at mid-podfill and involves biochemical changes in the plant and bacteroid. It is controlled primarily by nitrate availability, nodule age and plant maturation.

Gene Symbol, Chromosomal Location, Linkage data

Genes *Rj1*, *Rj2*, *Rj3*, *Rj4*, *Rj5*, *Rj6*, *Rj7*, and *Rj8* regulate nodulation in soybean. Rj2 has been mapped to 19a and 19b on the classical map and to linkage group J on the USDA RFLP map. Additional linkage data are detailed.

Mutants

Nonnodulating and hypernodulating mutants of soybean are referenced.

Source

The biological source of all cited research is specified. Any cultivar named in a study is listed, and these are "hot linked" to any other reference to that cultivar in SoyBase. For instance, if cultivar Bragg is clicked, a hypertext window shows that this cultivar is also a differential for Cowpea chlorotic mosaic virus, has been used for a study of 15 enzymes, 6 alleles, and 1 nodulin. All rhizobia identified in an experiment are listed, because they are integral to the nodulation process.

Regulation

Nodulation may be governed by developmental, hormonal, environmental, and genetic signals. Environmental control involves phenolics, nitrate, temperature, cations, light, O_2, and pathogens. Auxins are implicated in nodulation of legumes. Both plant and bacterial genetics may affect nodulation. References are cited for all methods of regulation.

The enzymes of biological nitrogen fixation are found within the Enzyme class of SoyBase. Nitrogenase is a complex enzyme comprised of two parts: dinitrogenase and dinitrogenase reductase. Ancillary enzymes are dinitrogenase reductase ADP-ribosyl transferase and dinitrogenase reductase-activating glycohydrolase. Reactions catalyzed by these enzymes are found by hypertext links to Reaction or Pathway.

Dinitrogenase

Dinitrogenase (E.C. 1.18.6.1) is also known as Component I or MoFe protein and has been cloned from *Bradyrhizobium* and other prokaryotic organisms. The many *nif* genes of *B. japonicum* are listed and referenced. The enzyme has been purified from soybean bacteroids, *Klebsiella*, *Azotobacter*, and others. It has a native molecular weight of 200-240 kD and is composed of 2 different polypeptides. Its 3D structure has been determined. As with all enzymes in SoyBase, biological sources are identified. Kinetics, inhibition, pH or temperature optima, and regulation data are available through hypertext links to the reaction catalyzed by dinitrogenase. Thirty references are cited describing environmental and genetic regulation of nitrogenase in soybean.

Dinitrogenase reductase

Dinitrogenase reductase (Component II or Fe protein) has been cloned, sequenced, and purified from several organisms. Five prokaryotic genes are involved, with *nifH* being the structural unit in B. rhizobium. It is a homodimeric protein with native molecular weight of 60-64 kD. It shows a high degree of sequence similarity in many organisms.

Dinitrogenase reductase ADP-ribosyltransferase
Dinitrogenase reductase-activating glycohydrolase

Dinitrogenase reductase ADP-ribosyltransferase and dinitrogenase reductase-activating glycohydrolase are post-translational regulatory proteins purified from *Rhodospirillum* and *Azospirillum*. At present it is unknown whether similar mechanisms operate in *Bradyrhizobium*. It has been determined, however, that the general functions of these two enzymes are very similar in the organisms from which they have been identified.

Access to SoyBase

Data regarding nodulation and dinitrogen fixation, as well as all other SoyBase data, may be accessed from the National Agricultural Library via the World Wide Web. The URL is http://probe.nalusda.gov:8000/plant/aboutsoybase.html. The SoyBase home page is located at http://macgrant.agron.iastate.edu. This page provides links to the WWW version of SoyBase and also contains graphical displays and references not included in SoyBase. A version of SoyBase for Macintosh is available upon request (dgrant@iastate.edu).

Acknowledgment

Prior to inclusion in SoyBase, data on nodulation and dinitrogen fixation were reviewed by Dr. John Imsande, Professor of Agronomy at Iowa State University.

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Rhizoctonia Foliar Blight Reactions in Soybean

Introduction

Rhizoctonia foliar blight (RFB) of soybean is caused by the fungus Rhizoctonia solani Kuhn anastomosis group (AG) 1. Yang et al. (1990) showed that RFB on soybean was caused by two types of *R. solani* AG1: intraspecific group A (IA), which causes aerial blight, and intraspecific group B (IB), which causes web blight. These types differ primarily in cultural characteristics and in morphology of sclerotia, which are the vegetative resting structures of the fungus. Field symptoms caused by these types are similar. The IA type of *R. solani* AG1 also causes sheath blight in rice, and rice-soybean double cropping may contribute to increased disease incidence on both crops. This disease has been observed in Louisiana since the early 50's and now affects most of the soybean acreage in south Louisiana. RFB also occurs in other states along the Gulf of Mexico and is important to soybean production in tropical and subtropical regions of the world.

RFB on soybean is difficult to control. Because labeled fungicides have limited effectiveness, most recommendations have focused on use of less-susceptible cultivars. However, reliable estimates of cultivar susceptibility have been difficult to obtain for several reasons. Disease distribution in a field frequently is uneven, thus allowing cultivars that merely escaped disease to be considered resistant. In addition, yield loss may be greater when the fungus attacks during flowering and early pod development stages than during later growth stages. Infection during later growth stages may result in visible foliar symptoms but minimal yield reduction, in some cases. Because of the increasing importance of RFB, reliable data on disease reactions of soybean cultivars and breeding genotypes are critical for ongoing efforts to develop disease resistance.

Most southern-grown soybean cultivars have been screened for RFB, but high aerial blight resistance has not been identified (Harville et al. 1996).

Our objective in this study was to screen the entire soybean germplasm base (Group IV through VII) in an attempt to find resistance to aerial blight that has not previously been identified.

Materials and Methods

In 1995 and 1996, approximately 4,500 Maturity Group (MG) IV through VII soybean germplasm lines were planted at Baton Rouge, Louisiana to evaluate their reaction to *Rhizoctonia* aerial blight. Plots were single rows ten feet long. Disease ratings range from 0 to 5, where 0 = no disease present and 5 = all plants within a plot infected, including some pod loss. Plots were artificially inoculated. Inoculum was prepared by culturing isolates BHIA-10 (*R. solani* AG1 IA) and BHMS-1 (*R. solani* AG1 IB) separately on potato dextrose agar at 30°C in darkness for 3-5 days. Cultures, consisting of fungus mycelium in agar, were combined with water and blended at high speed for 30 seconds. Suspensions of mycelium fragments were diluted with water to concentrations of 2×10^5 ml^{-1}. Equal amounts of suspensions from IA and IB were combined immediately prior to inoculations. Inoculum was applied at dusk with backpack sprayers. Genotypes in each maturity group were inoculated once during the R2-R3 soybean growth stages. Nozzles were positioned on the sprayers to direct inoculum both to the foliage and the stems.

RFB severity ratings were made when plants were at R5-R6, using a disease severity scale that included pods as well as foliage. The severity scale was: 0 = no disease, 1 = <5%, 2 = 6-10%, 3 = 11-30%, 4 = 31-50%, and 5 = >50% of pods and foliage with blight symptoms. This scale is purposely skewed toward lower disease severity to facilitate identification of the most resistant genotypes.

Results and Discussion

A range of reactions to RFB was observed among the germplasm, but few PIs demonstrated promising levels of disease resistance (Table 1). The vast majority of the PIs were highly susceptible to aerial blight, and only one entry, Kahala, was given a rating of 1. Forty-five lines (1%) were given a rating of 2. More than half of those lines given a rating of 2 were developed cultivars. Fifty-two percent of those cultivars were in maturity group VII. This suggests that indirect selection for resistance to RFB may have occurred as these lines were evaluated at sites

where the disease may have caused some reduction in seed yield.

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Table 1. Soybean PI's and cultivars that showed some possible resistance to aerial blight.

Strain	Cultivar	Maturity Group	Rating
	Kahala	IV	1
	Leflore	VI	2
	Rhodes	V	2
	Forrest	V	2
	Columbus	IV	2
	Bedford	V	2
	Crowley	V	2
	Doles	VI	2
	Essex	V	2
	Delsoy 4500	IV	2
PI 159095	41S31	VII	2
PI 200451	Amakusa Daizu	VII	2
PI 224270	Howaguchi	VII	2
PI 228065	Yamaguchi	VII	2
PI 230973		VII	2
PI 393545		VII	2
PI 399035		IV	2
PI 419043	Liyapa	IV	2
PI 430600 B	(Ta li chiang)	V	2
PI 430626	11238-2	V	2
PI 436567	Zan bian no. 20	VI	2
PI 437126 C	Imeretinscaja	V	2
PI 437726	Ta-jue-baj	VI	2
PI 468966	DH 4	VI	2
PI 476908	SO 82	V	2
PI 476921	Tuqui Xanh a	V	2
PI 507059	Mumei	VII	2
PI 518296		VI	2
PI 518665	Padre	VII	2
PI 518765	Centenaria	VII	2
PI 522236	Thomas	VII	2
PI 533602	Lloyd	VI	2
PI 533605	Cordell	V	2
PI 536009	Colquitt	VII	2
PI 548446	Charlee	VII	2
PI 548491	Tarheel Black	VII	2
PI 548496	Woods Yellow	VII	2
PI 548979	Govan	VII	2
PI 548989	Ransom	VII	2
PI 553042	Wright	VII	2
PI 553046	GaSoy 17	VII	2
PI 553047	Gordon	VII	2
PI 561372	Fendou No. 33	V	2
PI 567713 E	[Fu yang(36)]	IV	2
PI 572238	Haskell	VII	2
PI 88816 S	Hota	VI	2

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Allele contribution of parents to selected progeny from two-way crosses.

Introduction

Selection for improvement of genetic traits requires genetic variation. Genetic variation within a population of progeny from a cross pollination is a function of genetic diversity between the parents. Plant breeders often use coefficient of parentage as an estimate of the genetic diversity between potential parents. However, coefficient of parentage is a probability, and does not account for unbalanced contribution of alleles from parents as a result of selection for specific traits, environmental adaptation, or random drift. Analysis using molecular markers can provide genetic distance estimates unbiased by the assumption of equal contribution from the two parents, but requires investment in time and laboratory equipment. The extent to which accuracy is improved over coefficient of parentage is unknown.

Many high-yielding cultivars or experimental lines were selected from crosses between 'Williams', and 'Essex'. The number of selected lines from this parental combination provides a unique opportunity to evaluate the contribution of alleles from each of the parents in lines selected over a broad range of environments and breeding programs.

The cross Williams by 'Ransom' also resulted in a number of progeny released as cultivars from Dr. R. Cooper's soybean breeding program at Ohio State University. Progeny were selected using a modified early generation selection procedure described by Cooper (1990). Progeny were selected for high yield, lodging resistance, and determinate growth habit, with specific adaptation to highly productive environments.

Both crosses were between a northern (Williams, Maturity Group (MG) III) and a southern (Essex, MG V; or Ransom, MG VII) cultivar. Selections from the Williams by Essex crosses were made over a wide range in adaptive environments, while selection within the Williams by Ransom cross were made in a region suited for the maturity group of Williams, but for the semi-dwarf, determinate growth habit of Ransom.

Analysis of restriction fragment length polymorphism (RFLP) diversity among these groups of selected soybean lines provided an opportunity to examine whether deviation from coefficient of parentage was significant. The RFLP markers provided a sample of the allelic contributions from each of the parents and insight into the genetic consequences of selection.

Materials and Methods

The parent cultivars Williams, Essex, and Ransom, 10 cultivars selected from the cross Williams by Essex, and 5 from the cross Williams by Ransom were evaluated at 55 RFLP marker loci (a list of clone/enzyme combination is available upon request). Soybean DNA was extracted from greenhouse plants according to Keim and Shoemaker (1988) with modification. Southern blotting was done according to Maniatis et al. (1982) with adaptations described by Diers and Osborn (1994).

The soybean DNA was hybridized with 49 clones. The clones were obtained from Iowa State University and the University of Utah (Keim and Shoemaker, 1988). Clones were selected because they were previously shown to reveal a high frequency of polymorphism within elite germplasm (Webb, 1992; Skorupska, et al., 1993).

The probability of obtaining the sample allele distribution given an actual contribution of one-half of the alleles by each parent (coefficient of parentage = 0.5) was calculated from the binomial distribution, using the formula:

$$\frac{n(n-1)(n-2)\ldots(n-r-1)}{r(r-1)(r-2)\ldots(2)(1)}\ p^{r}(1-p)^{n-r}$$

where n is the number of segregating alleles sampled in the progeny, r is the sampled number of alleles from one of the parents (n - r being the number from the other parent), and p is the proportion of progeny having that allele in the infinite population from which the n samples were drawn (Snedecor and Cochran, 1967).

Cluster analysis was performed on the similarity matrix using the unweighted pair-group method, arithmetic average (UPGMA). Graphics were produced using NTSYS-pc software (Rohlf, 1992).

Results and Discussion

Although the coefficients of parentage were near zero between Williams and both Essex and Ransom (Carter et al., 1993), Williams differed from Essex at

only 17 of 55 loci examined and from Ransom at only 21 of 55. The cultivars 'NKB-335', 'P9471', 'A3127', and 'Pixie' differed significantly from an equal contribution of alleles from each parent (Table 1). When cultivars are grouped by company or university of origin, all groups, except for the cultivars developed by Asgrow differed significantly from an equal contribution by each parent. The Northrup King lines had significantly more Williams alleles, even though two of the lines shown in the table were actually acquired from different breeding programs (NKC-393 was formerly Coker 393, and NKB-335 was formerly Pride B335). The lines within this group may have been selected under similar environments. The Asgrow lines span maturity groups III, IV, and V; and may represent a broader range of adaptation than the other breeding programs. Grouping lines by maturity results in a significantly greater contribution of alleles from Ransom within lines from MG-IV (data not shown).

Cluster analysis of parents and progeny of the cross Williams by Essex (Fig. 1) shows most of the progeny associated with Williams. Grouping of lines implies common alleles are shared among them. The five maturity group (MG) III lines shared common alleles at 5 of 17 loci (pA89, pK14, pK385, pA203, and pR201). Four of these were Williams (MG III) alleles. At the locus where they shared an Essex allele (pA203), that allele was present in all the Williams by Essex progeny examined. No common alleles were shared by all MG IV progeny except the Essex allele (pA203) that was shared by all progeny. In 21 of 51 possible cases (3 breeding programs by 17 clones), Williams by Essex progeny within a breeding program all shared the same allele. Within breeding programs, common alleles were shared by all lines at 5 (Northrup King), 11 (Pioneer HI-Bred), and 5 (Asgrow) loci out of 17. It is likely that there is selection within breeding programs for traits associated with specific alleles.

Progeny of the cross Williams by Ransom were selected for high yield, lodging resistance, determinate growth habit, with specific adaptation to highly productive environments (Cooper, 1995). Progeny were selected using a modified early generation testing procedure (Cooper, 1990), which resulted in selection from within inbred lines. 'Elf', 'Gnome', and Pixie were selected from a common F_2 line, as were 'Sprite' and 'Hobbit' (Carter et al., 1993). Elf and Gnome were selections from within the same F_3 line (Cooper and Martin, 1981) and the dissociation of Elf and Gnome in the cluster analysis (Fig. 2) is likely an artifact from the limited number of loci examined and the close relationship among all of the selections. Cluster analysis of Williams, Ransom, and progeny (Fig. 2) shows greater association of progeny with Ransom than Williams. This is contrary to expectations based on adaptation to maturity environments. The range of maturity groups in the progeny was from MG II to MG IV; more similar to Williams (MG III) than to Ransom (MG VII). This shows that favorable gene combinations can be introgressed from gene pools outside areas of adaptation , especially in conjunction with selection for specific traits such as determinate growth habit.

Conclusions

Selection within breeding programs for adaptation to particular growing areas or specific traits can result in a significant deviation from genetic relationships estimated by the coefficient of parentage. For this reason, distance coefficients from RFLP analysis are probably more accurate than those from coefficient of parentage, even when pedigree information is available and accurate.

The association of progeny with a parent outside their maturity group in selection for specific traits, such as semi-dwarf, determinate genotypes, shows that unadapted germplasm can be a source for new, favorable gene combinations.

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Table 1. Contribution of alleles from parent cultivars to selected progeny of the crosses Williams (MG III) by Essex (MG V) and Williams by Ransom (MG VII).

Williams by Essex

Progeny Name	Williams alleles		Essex alleles		
	Number	Percent	Number	Percent	Prob.†
NKC-393(III)	11	65	6	35	0.09
NKB-335(III)	12	71	5	29	0.05*
NKS42-40(IV)	10	59	7	41	0.15
Total Northrup King	33	65	18	35	0.01**
P9441(IV)	6	35	11	65	0.09
P9471(IV)	5	29	12	71	0.05*
Total Pioneer	11	32	23	68	0.02*
A3127(III)	5	29	12	71	0.05*
A3860(III)	8	47	9	53	0.19
A3966(III)	10	59	7	41	0.15
A4268(IV)	10	59	7	41	0.15
A5308(V)	11	65	6	35	0.09
Total Asgrow	44	52	41	48	0.08

Williams by Ransom

Progeny	Williams alleles		Ransom alleles		
	Number	Percent	Number	Percent	Prob.
Gnome(II)	9	43	12	57	0.14
Elf(III)	9	43	12	57	0.14
Pixie(IV)	5	24	16	76	0.01**
Sprite(III)	8	38	13	62	0.10
Hobbit(III)	8	38	13	62	0.10
Total Ohio St. Univ.	39	37	66	63	<0.01**

† The probability value is for the allele distributions given and is calculated from the binomial frequency distribution assuming the null hypothesis of allele frequencies of 0.5. *,** significant at .05 and .01 levels, respectively. Maturity groups are given in parentheses.

Figure 1. Cluster analysis of the parents and progeny of the cross Williams by Essex.

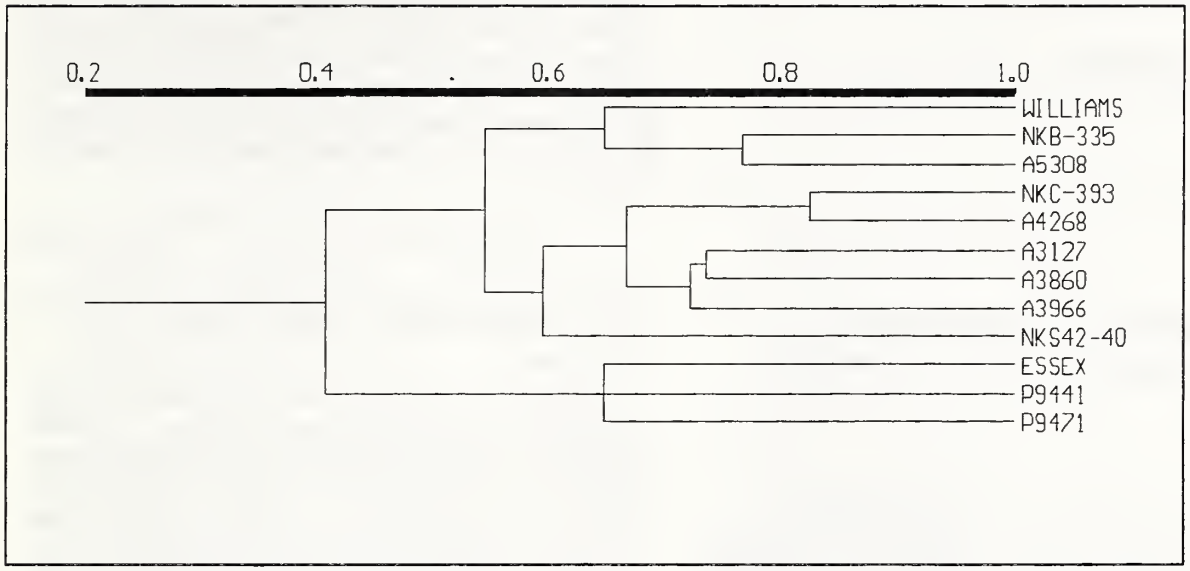
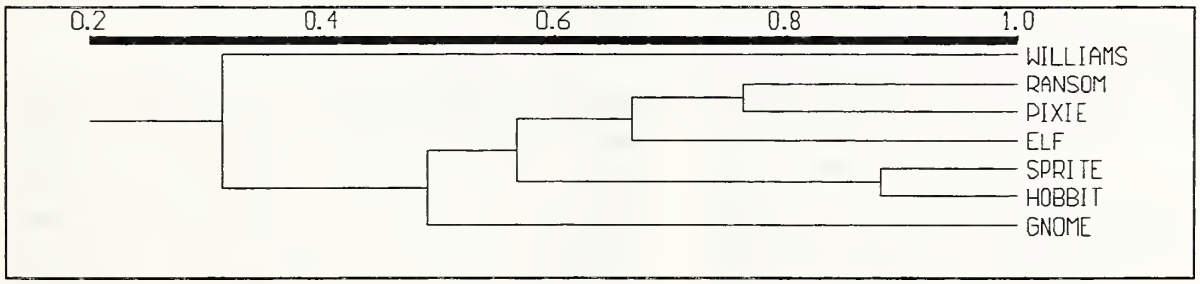


Figure 2. Cluster analysis of the parents and progeny of the cross Williams by Ransom.



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Genetic Mapping of Soybean Cyst Nematode Resistance Genes from PI 88788

PI 88788 is one of the most widely used sources of resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN). Many high yielding soybean [*Glycine max* (L.) Merr.] cultivars are currently available to growers that have resistance derived from this plant introduction (PI). Classical genetic studies indicate that Race 3 SCN resistance in PI 88788 is conditioned by two dominant and one recessive genes (Rao-Arelli et al., 1988). Further research suggested that one of the dominant genes is *Rhg4*, which maps close to the *i* gene (Matson and Williams, 1965), and the recessive gene is *rhg2* (Rao-Arelli et al., 1992).

Concibido et al. (1997) mapped a gene from PI 88788 that provides resistance to SCN Races 3 and 6 to linkage group 'G'. This is the same region that a major resistance locus was mapped in PI 437654 (Webb et al., 1995) 'Peking', PI 90763, and PI 209332 (Concibido et al., 1996; Concibido et al., 1997). The objective of our research was to confirm the mapping of the resistance gene on linkage group G and to map other genes from PI 88788 conferring resistance to SCN Races 3 and 14.

Materials and Methods

The genetic mapping was done in a population derived from a cross between the cultivars Bell (Nickell et al., 1990) and Colfax (Graef et al., 1994). Bell has resistance to SCN Races 3 and 14 derived from PI 88788 and Colfax is susceptible to SCN. Crosses were made between these parents in 1993, and two F_1 plants were grown in 1994. F_2 and F_3 plants from the crosses were advanced by single-seed descent in Belize during the winter of 1994-1995 and F_4 plants were grown in the field in Michigan during the summer of 1995. The F_4 plants were harvested and threshed individually to form F_4 derived lines. Fifty-four $F_{4:5}$ lines derived from one F_1 plant and 49 $F_{4:5}$ lines derived from the second F_1 plant were evaluated for resistance to SCN Races 3 and 14. Five plants from each line were tested for SCN resistance using procedures outlined by Diers et al. (In press). An index of parasitism (IP) was calculated for each plant using the formula (Golden et al., 1970).

$$IP = \frac{\text{Average number of cysts and females per accession}}{\text{Average number of cysts and females per Hutcheson}} \times 100$$

DNA was extracted from approximately ten greenhouse grown plants from each line. The DNA extraction, Southern blotting, and hybridizations were done according to Kisha et al. (1997). Associations between the segregation of genetic markers and IP were tested using one-way analyses of variance for each marker race combination. Linkage among the markers was determined using the program MAPMAKER/EXP (Lander et al., 1987).

Results and Discussions

Lines from the populations were evaluated for resistance to SCN Races 3 and 14. The IP of lines for Race 3 ranged from 3 to 125 with a mean of 53. The IP of lines for Race 14 ranged from 3 to 165 with a mean of 57. The Race 3 and 14 IP of lines had a correlation of 0.68.

The populations were evaluated for 15 restriction fragment length polymorphism (RFLP) marker loci. Markers mapping to two linkage groups were significantly ($Pr<0.01$) associated with both Race 3 and 14 resistance (Table 1). The marker alleles from Bell, the SCN resistant parent, were associated with greater resistance than alleles from Colfax for each significant marker. The marker with the greatest association to resistance is Bng122, which is on linkage group 'G' (Concibido et al., 1997). The R^2 value for Bng122D is 0.65 for Race 3 and 0.43 for Race 14. K069I, a second marker on linkage group G, is also significantly associated with SCN resistance. The linkage distance between Bng122D and K069I is 22 cM and these markers presumably are linked to the same resistance gene or cluster of genes. The association between SCN resistance and linkage group G is consistent with findings of Concibido et al. (1996, 1997). They found the same region on linkage group G from PI 88788, PI 209332, PI 90763 and Peking to be associated with SCN resistance.

The second marker significantly associated with SCN resistance is A063I, which was previously mapped to linkage group 'C' (Lorenzen, et al., 1995). A063I is significantly associated with resistance to both Race 3 and 14. Further work needs to be done to confirm that the polymorphism we have mapped for A063 is on linkage group C.

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Table 1. Markers significantly associated with resistance to SCN Races 3 and 14 in the cross Bell by Colfax.

Marker	Linkage Group	Race	Pr> F	R^2	BB†	CC‡
K069I	G	3	0.0001	16.9	34	61
K069I	G	14	0.0002	16.1	38	66
Bng122D	G	3	0.0001	65.3	18	78
Bng122D	G	14	0.0001	43.0	27	80
A063I	C	3	0.001	12.9	40	68
A063I	C	14	0.001	11.6	44	72

† Mean of the lines homozygous for the allele from the resistant cultivar Bell.

‡ Mean of the lines homozygous for the allele from the susceptible cultivar Colfax.

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Construction and characterization of a soybean bacterial artificial chromosome (BAC) library

Introduction

Large DNA insert libraries are extremely valuable for analyzing genome organization and as tools for positional gene cloning. We are interested in the genes involved in soybean-pathogen interactions, especially those that confer resistance to soybean cyst nematode (*Heterodera glycines*). As a foundation for map-based cloning, we have constructed a bacterial artificial chromosome (BAC) library for soybean using DNA from the cyst nematode resistant variety, 'Faribault'. This library provides a threefold redundant representation of the soybean genome with an average insert size of 120 kilobases (kb). Detailed analysis of a small number of clones indicates the library will be useful for physical mapping, chromosome walking, and gene cloning experiments.

Materials and Methods

Partial digestion and size selection of megabase-sized DNA. Cotyledonary leaves of 10 day old Faribault plants were harvested and used for preparation of megabase-sized DNA in low melting point agarose plugs (Danesh et al., 1994). A 60 µl agarose plug containing about 6.0 µg DNA was incubated in*Eco*RI reaction buffer on ice for 60 min, which was replaced with fresh buffer containing 2 to 3 units of *Eco*RI enzyme and incubated on ice for another 60 min. Partial digestion was performed by transferring the plug to a 37°C water bath for 5 min, and stopped by adding 0.1X volume 0.5 M EDTA, pH 8.0. Partially digested DNA was separated on a 1% low melting point agarose gel using a clamped homogeneous electric field (CHEF) DRII apparatus. The DNA was separated in TAE buffer at 10°C, 6.0 volts/cm with 90 s pulse time for 20 h. DNA fractions ranging in size from 225-285 kb, 285-365 kb, and 365-450 kb were excised from the gel and used in ligation reactions.

BAC vector preparation. *pECS*BAC4, a BAC vector 9.3 kb in size containing a unique *Eco*RI restriction site for fragment insertion, was kindly provided by Dr. Richard Michelmore (University of California, Davis). For preparation of the vector, the protocol of Woo et al. (1994) was followed with the following modifications. After QIAGEN plasmid Maxi-kit purification and ethanol precipitation, DNA was re-suspended in 1X TA buffer (33 mM Tris-acetate, pH 7.8, 66 mM K-acetate, 10 mM Mg-acetate, 0.5 mM DTT). Plasmid DNA (10 µg) was digested to completion with *Eco*RI at 37°C for four hours. After verifying plasmid digestion on an agarose gel, the *Eco*RI was heat-inactivated. DNA was precipitated with ethanol and re-suspended in 60 µl 1X TA buffer plus 5 mM $CaCl_2$. Plasmid DNA was dephosphorylated using HK phosphatase (Woo et al., 1994). The extent and integrity of dephosphorylated DNA was assayed by self-ligation. One µg aliquots of dephosphorylated vector DNA were stored at -80°C until used.

BAC library construction. Partially *Eco*RI digested soybean DNA was dialyzed against TE (pH 8) on ice for 60 min and used in ligation reactions with *Eco*RI-digested and -dephosphorylated vector DNA according to Woo et al. (1994). The ligation reaction was then subjected to drop dialysis for two hours at room temperature. One µl of the dialyzed ligation reaction was used to transform 20 µl of *E. coli* Electro Max DH10B cells (BRL) by electroporation using an ECM 600 electroporator machine (BTX, Inc.). Plating of transformed cells and selection of recombinant white colonies followed standard protocols (Wang et al., 1995; Woo et al., 1994). White recombinant BAC clones were picked manually and transferred to 384 well plates (Nalge-Nunc) containing 70 µl of LB freezing medium with 12.5 µg/ml chloramphenicol (Wang et al., 1995). Plates were wrapped in plastic and incubated at 37°C overnight or up to 24 hours, when sufficient bacterial growth was evident. Plates were then placed at -80°C for long term storage. The library was replicated three times and stored in three different freezers.

Characterization of the BAC clones. For insert isolation, BAC clones were shaken in LB medium with chloramphenicol for 20 hours at 37°C and a standard alkaline lysis protocol was followed (Silhavy et al. 1984). Inserts were excised with *Not* I and resolved on a CHEF gel.

Preparation of high density filters. Each Hybond N^+ filter (115 X 75 mm, Amersham, USA) was inoculated with the bacterial clones of four 384 well plates (a total of 1536 clones) using the Nunc

replication system. The inoculated filters were placed on Nunc OmniTrays containing LB agar with 12.5 µg/ml chloramphenicol and incubated at 37°C overnight until colonies were approximately 2-3 mm in diameter. The library of 30,720 BAC clones was blotted onto 20 high density filters with two replications. Processing of filters was performed as described (Woo et al., 1994) and probing high density filters (as well as dot-blots prepared in the course of the research) with radiolabeled DNA markers was as previously reported (Danesh et al., 1994).

Preparation of pools and sub-pools of BAC DNA. In the preparation of a DNA pool, BAC clones from four 384 well plates (1536 BAC clones) were replicated onto a Nunc OmniTray containing LB agar with 12.5 µg/ml chloramphenicol using the Nunc replication system. For sub-pool DNA preparation, only 384 BAC clones from a single plate were replicated in a similar manner. Trays were incubated at 37°C until colonies had grown to 2-3 mm in diameter. To prepare the DNA pools and sub-pools, all colonies in a single tray were suspended in 20 ml sterile water and transferred into a 50 ml sterile tube. Cells were precipitated by centrifugation and DNA isolated by an alkaline lysis protocol (Silhavy et al., 1984). The final DNA pellet was resuspended in 0.5 ml TE. A total of 20 DNA pools and 80 sub-pools were prepared, with the concentration of DNA in the pools ranging from 10 to 130 µg/ml.

BAC-End Isolation. Separate aliquots of BAC DNA were digested with *Bam*HI, *Hind*III, or *Sph*I in TA buffer. After heat inactivation of the enzymes (65°C/15 min), the digested DNA was self-ligated at 16°C for 2-3 h. One to two µl of the ligation reaction was used in a 50 µl polymerase chain reaction (PCR) reaction. Primers AB3 and AB4 (Sequence kindly provided by David Baulcombe, Sainsbury Laboratory, U.K.) were used for some left end isolations, whereas T7 and BAC 4 (Woo et al., 1994) were used for amplification of right (*Bam* HI digest) or left BAC ends (*Hind*III digest). PCR reactions were performed on a PTC100 Thermal Controller (MJ Research), under conditions reported by Cai et al. (1995).

Results and Discussion

Construction of the BAC library. The plasmid *pECS*BAC4 has three unique cloning sites within the *LacZ* gene, *Bam*HI, *Eco*RI, and *Hind*III. Previous BAC vectors lacked the unique *Eco*RI site. The library was constructed using the *Eco*RI site because *Eco*RI is a reliable endonuclease that can be used in combination with *Eco*RI methylase. It also makes the BAC system compatible with *Rec*A assisted restriction endonuclease and amplified fragment length polymorphism (AFLP) markers. A small portion of the library (2.5%) was constructed using the *Hind*III cloning site of *pBelo*BACII. These clones will not be discussed here.

It has been reported that electroporation conditions of competent *E. coli* cells can lead to selective transformation of smaller or larger BAC clones (Sheng et al., 1995). The electroporation settings used in this study were based on the manufacturer recommendations of the ECS600 electroporator machine. The settings may have led to transformation of smaller recombinant BAC clones into cells and resulted in construction of a library smaller in size than might have been possible under ideal conditions (Sheng et al., 1995).

Importance of Freshly Dephosphorylated DNA. Preliminary experiments with dephosphorylated vector stored at -80°C produced satisfactory numbers of recombinant clones. However, subsequent ligation reactions and transformations showed a gradual decline in the number of recombinant BAC clones until finally no recombinant BACs could be generated. Self ligation of five week old dephosphorylated vector DNA (stored at -80°C) using T4 ligase and T4 kinase failed to generate distinct circular plasmid bands on an agarose gel. However, ligation of size selected partially *Eco*RI digested soybean DNA into freshly dephosphorylated plasmid vector followed by transformation led to production of recombinant BAC clones. We concluded that dephosphorylation might be leading to a rapid degradation of the plasmid DNA under our conditions. It is even possible that the difficulty in constructing BAC libraries in some labs could be due to degradation of dephosphorylated vector. It is recommended that dephosphorylated vector DNA be used fresh and long-term storage avoided.

The BAC library. The entire BAC library consists of approximately three soybean genome equivalents, containing 30,720 clones with an average insert size of 120 kb. Insert size, based on an analysis of 50 random BAC clones, ranged from 40 to 230 kb. Attempts to increase the average insert size using a second round of size-selection were unsuccessful. Therefore, first size selected partially *Eco*RI digested soybean DNA was used for the construction of the library.

Estimating the level of repetitive sequences. To estimate the level of repetitive sequences contained within BAC clones, dot blots prepared from 40 individual BAC DNA minipreps were hybridized with radiolabeled soybean genomic DNA. Those colonies that produced no signal after hybridization were inferred to be low copy; colonies with faint signals to contain some medium repetitive sequences, and

those with bright signals to contain some highly repetitive sequences. Of the colonies tested, 20% were found to be entirely low copy DNA, 25% contained some medium repetitive DNA, and 55% contained highly repetitive sequences.

Identification of BAC clones corresponding to specific RFLP markers. Four RFLP probes (C006, B053, D140, and Bng122) that map near the major cyst nematode resistance gene on linkage group 'G' were used to screen high density filters of the BAC library. For each of the clones, at least one corresponding BAC clone was identified. In the case of B053, three corresponding BACs were identified. Four of these BACs (one each for C006 and Bng122, two for B053) were subsequently shown to be located on linkage group G. The map location of the BAC corresponding to D140 has not yet been established. Based on DNA hybridization, two of these BACs consist exclusively of low copy DNA, one contains some medium repetitive DNA, and the other contains high copy DNA sequences.

End-cloning from BACs. In order to carry out chromosome walking and extend contigs that might be useful in positional cloning, we tested the library for PCR amplification of ends of isolated BAC clones. Specifically, we modified the method of Woo et al. (1994) for isolating right ends without a need for plasmid rescue. Another advantage of this system is its ease and speed. As much as 5.0 ng of BAC DNA can be subjected to different enzymatic digestion and modification in a single reaction without the need for DNA precipitation.

Using this cloning strategy, we successfully isolated left and right ends from two of the BACs described above. A combination of physical and genetic mapping of the ends for one of these BACs has demonstrated the clone was not rearranged. Subsequent screening of high density filters using the PCR amplified ends has uncovered two additional BACs that are physically linked to those already isolated. Detailed analysis of these new BACs is currently underway.

Polymerase chain reaction screening. PCR-based screening of BAC DNA pools using simple sequence repeats and sequence tag sites is also underway (Green et al., 1990). The results indicate that pools of BACs are suitable for PCR-amplification as a basis for screening the library. Optimizing the DNA concentration of the pools in order to give reproducible amplification has been essential, as PCR amplification tended to be inconsistent if template concentrations were too high. The BAC clones uncovered by this method, however, have not yet been characterized in detail.

Requests to screen the BAC library. Researchers who wish to screen this BAC library for clones of interest should contact Dr. Nevin Dale Young at e-mail neviny@puccini.crl.umn.edu or fax 612-625-9728. We anticipate that high density filters and pools derived from the library should be available in the next year.

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Identification of a Soybean Breeding Line Resistant to Rust in the Philippines

Introduction

Soybean rust (caused by *Phakopsora pachvrhizi* Syd.) has not been recognized in the continental USA, but concern over its potential for damage to the soybean crop has increased since the disease was identified in Hawaii in 1994.

Four genes conferring resistance to soybean rust have been identified. Hartwig (1986) reported on the resistance gene *Rpp4* found in PI459025, and summarized the rust reaction of the other three genes. Gene *Rpp4* confers resistance to rust isolate Taiwan 80-2, whereas genes *Rpp1*, *Rpp2*, and *Rpp3* were susceptible. These studies were conducted in containment facilities at the Foreign Disease and Weed Science Research Unit, Frederick, MD. The objective of this study was to transfer the gene *Rpp4* from the primitive germplasm line PI459025 to a better adapted genotype.

Materials and Methods

The cross Hardee X PI459025 was made at Stoneville, MS in 1984. F_2 seedlings from this cross were grown in a greenhouse at Frederick, MD, and inoculated with rust isolates. Resistant plants were grown to maturity and seeds were harvested from individual plants. In 1987, an advanced rust resistant F_3 line was used as a male parent in a cross with the variety Lamar. Some of the seed from F_2 plants from that cross was planted at Frederick and evaluated for soybean rust. Remnant seed from the same F_2 plants was planted at Stoneville. Twenty-five lines uniformly resistant to rust were advanced to the F_5 generation at Stoneville. During advance, Dr. E. E. Hartwig selected lines resistant to bacterial pustule, lodging, and seed shattering. Four lines (D91-5987, -5989, -5996, and -6023) were chosen for seed yield evaluation at Stoneville in 1992, 1993, and 1994. Dr. Hartwig requested that these four lines and D86-8286 (*Rpp3*) be planted in the Pioneer Hi-Bred Int'l 1995-96 winter nursery in the Philippines. Entries in the rust evaluation nursery were planted in a RCB design with four replications. Disease developed from a natural infestation. Visual ratings were assigned on a 1 to 9 scale, where 1 = severe disease symptoms, and 9 = no observable symptoms.

Results and Discussion

During the three years of replicated seed yield evaluation of Maturity Group VII lines at Stoneville, the best yielding line (D91-5987) averaged about 80% of the seed yield of the variety Braxton. Rust reaction to the four experimental lines, D86-8286 and some selected Pioneer lines is summarized in Table 1. Line D91-5987 had the least disease with a rating of 7.5, whereas the most severely diseased lines were rated a 2.5. The soybean rust isolate found in the Philippines is thought to be the same as the Hawaii isolate. This is being investigated by Dr. Morris Bonde, USDA-ARS, Frederic, MD. When Dr. Bonde's research is completed, we plan to release D91-5987 as a Maturity Group VII rust resistant germplasm line.

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Table 1. Reaction of soybean to a natural infestation of soybean rust in the Philippines

Entry	Rust reaction[a]	Rust resistant donor
D91-5987(*Rpp4*)	7.5	PI459025
D91-6023	5.0	PI459025
D91-5996	4.8	PI459025
P9611	4.8	
D91-5989	4.5	PI459025
P9501	4.3	
D86-8286(*Rpp3*)	3.0	PI230970
P9692	2.5	

[a] Rating of 1 = severe disease symptoms, 9 = no disease symptoms.

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Molecular Genetic Diversity Among Soybean Plant Introductions With Resistance To *Heterodera glycines*

Introduction

Planting a resistant cultivar to soybean cyst nematode (SCN), *Heterodera glycines,* causes a decrease in nematode population density, but it also causes selection pressure on nematode populations in the field and is responsible for genetic shifts of nematode races. Therefore, resistant cultivars containing several resistance genes will be required to maintain a low population density of SCN in the field for higher soybean yields. However in USA, improved soybean cultivars have derived their SCN resistance from only a few sources, *i.e.*, 'Peking', PI 88788 and 'Hartwig' (Rao Arelli, 1994). Additional germplasm is available which could be used as novel sources of resistance for broadening the genetic diversity of resistance (Anand et al., 1985, Young, 1990, and Rao Arelli et al., 1992). These resistant germplasm lines are mostly primitive cultivars and wild relatives, and do not have adequate pedigree information to determine their genetic relationships. Prior knowledge of the genetic relationships among these germplasm lines would facilitate development of novel resistance genes in soybean cultivars and lead to improved genetic diversity and gene pyramiding. Traditional laboratory techniques do not offer adequate tools for establishing their relationships.

The objective of our research was to use RFLPs to characterize genetic diversity of SCN resistant soybean germplasm lines.

Materials and Methods

In this study, fifty-two soybean PI lines and genotypes resistant to SCN and four susceptible controls were examined for genetic diversity among them. These PI lines were introduced at different times representing different geographic regions in the world. They belong to six different maturity groups and nearly two-thirds of them are black seeded (Table 1).

Genomic DNA was isolated from leaves of greenhouse-grown plants using standard methods (Shure et al., 1983), and was restriction digested using *Eco R I, Eco R V, Hind III, Dra I* and *Taq $^{\alpha}$ I.* Each restriction was size fractionated by electrophoresis and transferred to Magnacharge Nylon membrane for hybridization.

One-hundred and two soybean genomic probes covering 23 soybean linkage groups (developed by R. C. Shoemaker, USDA-ARS, Iowa State University and purchased from Biogenetic Services, Inc., Brookings, SD) were hybridized to the DNA membrane (UMC Maize RFLP Laboratory. January, 1995.). Polymorphisms were scored as plus (+, presence), minus (-, absence), and dot (., missing). Data were analyzed for dissimilarities between RFLPs among PI lines by Ward's minimum variance clustering method (7).

Results and Discussion

From 510 probe/enzyme combinations, 1707 hybridization bands appeared and 501 bands were scored as RFLPs. The polymorphism frequency was 29.3%. Clustering analysis, based on these data, established a dendrogram of 56 soybean genotypes (Fig. 1). The dendrogam showed two major clusters in which several sub-clusters were included. One major cluster contained all PI lines from South Korea (except one), the other major cluster included most PI lines from China; while the PIs from Japan, Russia and other countries were separated into two major clusters.

Four soybean cultivars, *i.e.*, 'Hartwig', 'Hill', 'Hutcheson', and 'Essex', for which their pedigree information was available (Fig. 2), were clustered together on the top of the dendrogram based on their genetic homology. Original ancestors of these cultivars were mainly from Korea, Northeast China and Japan (8) and included 'Haberland', 'Dunfield', 'Illini', 'Clemson', 'Patoka', 'Sato', 'Tokyo', PI54610, 'Roanoke' and 'Arksoy'. These ancestors also represented wild germplasm and were grouped with other PI lines mainly from Korea, South Korea, and North East China.

PI's country of origin seems to have played a role in clustering patterns. Based on this, the resulting two large groups included Korea and Northeast China as one group, and the other included mostly China.

In the cluster representing the China region several sub-groups were observed which included almost all the important SCN resistant PI lines which are mostly used in current breeding programs for introgressing resistance genes. Each of these sub-groups included a SCN resistant differential. They included Peking in one sub-group; PI 90763 and PI 88788 were placed in another; and PI 437654 belonged to the third sub-group (Table 1).

The dendrogram also suggested a strong relationship between geographic origin and SCN resistance of soybean. Most PIs from Korea were moderately resistant to SCN race 3. PIs in the Peking sub-group were found resistant to more than three races, *i.e.*, 1, 2, 3, and 5. The sub-group representing PI 437654 was resistant to moderately resistant to race 14 (except PI 404198A) plus at least to other two races. And PIs in the PI 88788 group were mainly resistant to SCN race 3, except PI 88788 itself which was resistant to SCN race 14. Other PIs in this sub-group were predominantly moderately resistant (except PI 90763). A detailed analysis is being conducted.

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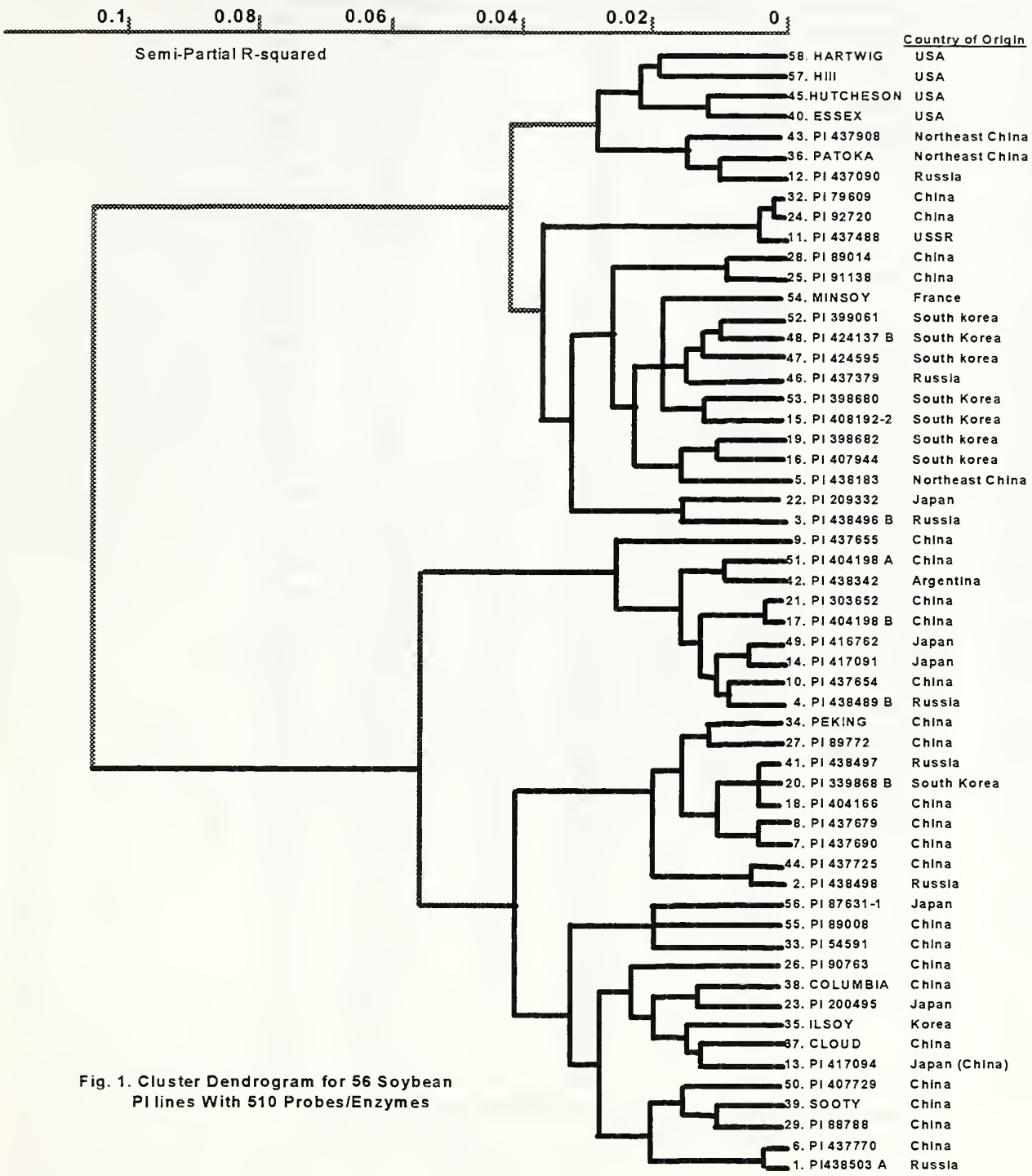

Fig. 1. Cluster Dendrogram for 56 Soybean PI lines With 510 Probes/Enzymes

Table 1. Characteristics of 52 resistant genotypes plus four susceptible controls

Plant Introductions	Cultivar or Accession	Country of Origin	Year Introduced	Maturity Group	R/MR/MS/S*	Seed Color
58.HARTWIG	Modern Cultivar	USA	1992	V	R	Yellow
57. Hill	Modern Cultivar	USA	1959	V	S	Yellow
45. HUTCHESON	Modern Cultivar	USA	1988	V	S	Yellow
40. ESSEX	Modern Cultivar	USA	1972	V	S	Yellow
43. PI 437908	Hsiao-hei-chin Eco	Northeast China	1980	II	MR 3	Mottle
36. PATOKA	PI 70218-2-19-3	Northeast China	1926	IV	MS 14	Yellow
12. PI 437090	DV-206	Russia	1980	O	MS 5,14	Yellow
32. PI 79609	N247/1250	China	1928	II	MR 3	Black
24. PI 92720	7915	China	1931	III	MR 3	Black
11. PI 437488	1070	USSR (China)	1980	II	MR 14	Black
28. PI 89014	5959	China	1930	II	MS 1,3,5,14	Yellow
25. PI 91138	6593	China	1930	II	MS 1,3,5,14	Y+Br
54. PI 27890	Old Cultivar (Minsoy)	France	1910	II	S	Yellow
52. PI 399061	-----	South Korea	1972	VI	R 5,MR 3	Yellow
48. PI 424137 B	Gunwi Gun	South Korea	1973	V	R 5,MR 14	Yellow
47. PI 424595	Yeongju Gun	South Korea	1978	VI	R 5	Black
46. PI 437379	Ussurijscaja 431	Russia	1980	I	MS 5	Yellow
53. PI 398680	Jochiweon Gun	South Korea	1975	IV	MR 3	Green
15. PI 408192-2	Gyeongju Gun	South Korea	1976	V	MS 1,5,14	Green
19. PI 398682	Jochiweon Gun	South Korea	1975	IV	MR 3	Black
16. PI 407944	Jeonju Gun	South Korea	1976	V	MR 3	Yellow
5. PI 438183	4447	Northeast China	1980	II	MR 3	Brown
22. PI 209332	crossed for forage	Japan	1953	IV	R 3,5,14	Black
3. PI 438496 B	Peking	Russia	1980	III	R 3, MR 1	Black
9. PI 437655	Er-huan-jan	China	1980	III	R 1,3,5, MR 14	Black
51. PI 404198 A	Sun-huan-do	China	1975	IV	R 1,2,3,5	Black
42. PI 438342	Laredo J 767	Argentina	1980	VI	R 5, MR 2,14	Bl+Y
21. PI 303652	Lian-zia-tno	China	1965	V	R 1,3,5, MR 14	Black
17. PI 404198 B	Sun-huan-do	China	1975	IV	R 1,3,5, MR 14	Black
49. PI 416762	Akanida	Japan	1974	II	R 3,5,14, MR 1	Black
14. PI 417091	Kuro Mame	Japan	1977	II	R 3,5,14, MR 1	Black
10. PI 437654	Er-hei-jan	China	1980	III	R 1,2,3,5,14	Black
4. PI 438489 B	Chiquita	Russia	1980	IV	R 1,2,3,5,14	Black
34. PEKING	17852B	China	1906	IV	R 1,3,5	Black
27. PI 89772	7193	China	1930	IV	R 1,2,3,5	Black
41. PI 438497	Peking-206	Russia	1980	III	R 1,5	Black
20. PI 339868 B	Yuwoltae	South Korea	1969	IV	R 1,3,5	Black
18. PI 404166	Krasnoarmejskaja	China	1975	III	R 1,2,3,5	Black
8. PI 437679	Nan-cou	China	1980	IV	R 3,5,14, MR 1,2	Black
7. PI 437690	Pin-din-guan	China	1980	III	R 1,2,3,5, MR 14	Black
44. PI 437725	Te-zu-gan	China	1980	IV	R 1,3,5, MR 2	Black
2. PI 438498	Sable	Russia	1980	IV	R 1,3,5	Black
56. PI 87631-1	5640-Kindaizu	Japan	1930	III	R 3, MR 5,14	Mottle
55. PI 89008	5953	China	1930	II	MR 3,5,14	Y+Bl
33. PI 54591	31	China	1921	III	MR 5	Y+Br
26. PI 90763	7570	China	1930	IV	R 1,2,3,5	Black
38. COLUMBIA	PI 22897	China	1908	III	R 3,14, MR 1,5	Green
23. PI 200495	Kuro Daizu	Japan	1952	IV	R 5, MR 1,3,14	Black
35. ILSOY	13-9	North Korea	1901	III	MR 1,3,5,14	Brown
37. CLOUD	PI 16790	China	1905	III	R 3, MR 14	Black
13. PI 417094	Kuro Masshokutou	Japan (China)	1977	III	MR 3,5,14	Black
50. PI 407729	------	China	1974	IV	R 5,14, MR 1,3	Black
39. SOOTY	------	China	1907	IV	MR 3,5,14	Black
29. PI 88788	5913	China	1930	III	R 3,14	Black
6. PI 437770	1278	China	1980	III	R 3, MR 14	Black
1. PI438503 A	Wu Dow	Russia	1980	II	R 3,14, MR 5	Black

* R = Resistant (Index of Parasitism = 0-9%), MR = Moderately Resistant (IP = 10-30%)
MS = Moderately Susceptible (IP = 31-60%), S = Susceptible (IP > 60%)

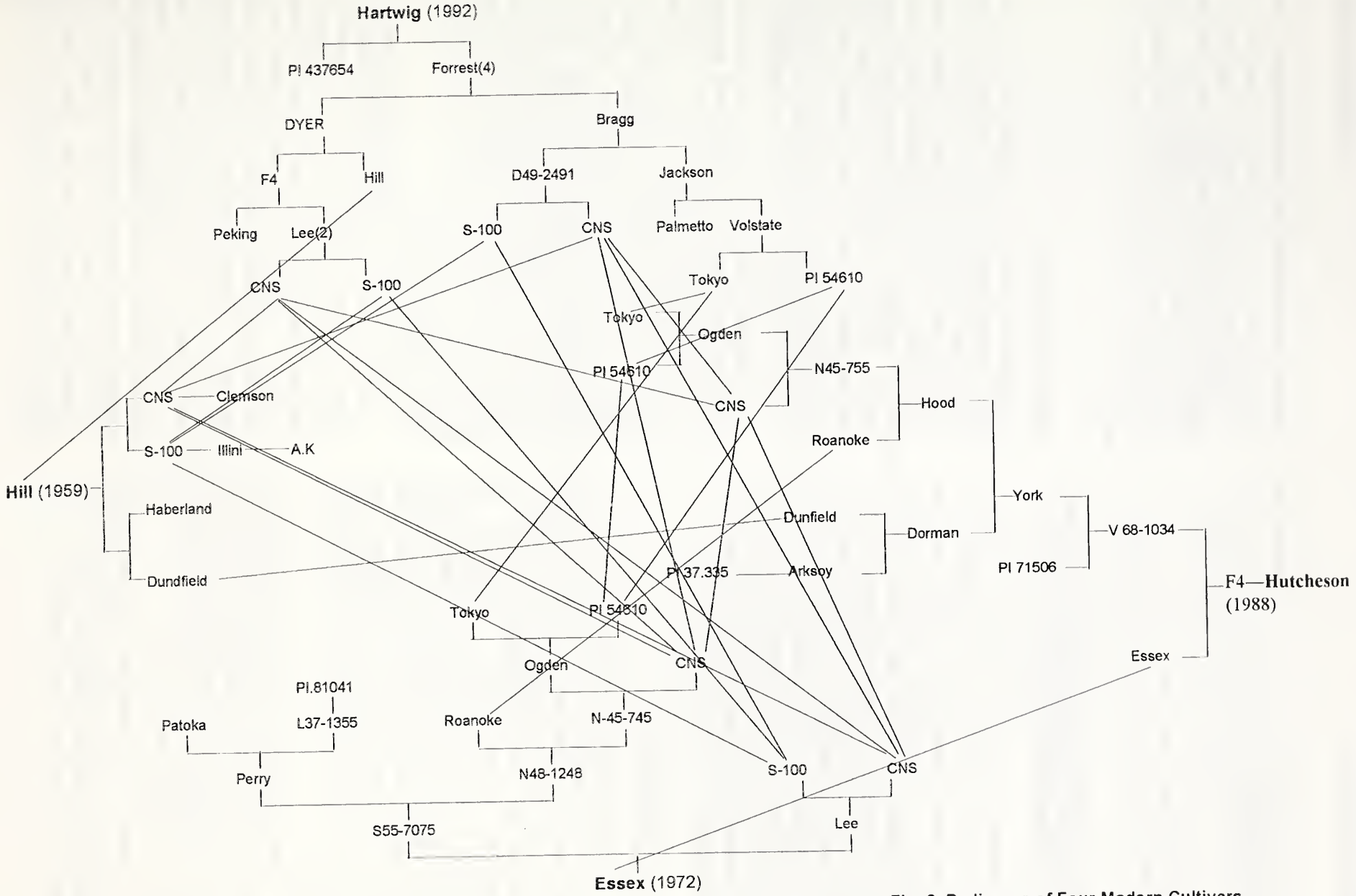

Fig. 2. Pedigrees of Four Modern Cultivars

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RFLP Markers Associated With Soybean Oil And Protein Concentration

Introduction

Soybean seed oil and protein concentrations are polygenic traits (Burton et al., 1987), and are influenced by environmental factors. Molecular markers used as an indirect selection tool can be used for characterizing quantitative trait loci (QTL), and simplifying the genetic analysis of quantitative traits. However, only limited information is available on the association of DNA markers and soybean protein or oil concentration (Mansur et al., 1993; Lark et al., 1994). Objective of this study was to identify DNA markers which were linked to loci controlling soybean seed oil and protein concentrations.

Materials and Methods

Two parents, 'Peking' and 'Essex', were used to generate F_1 hybrids, which were used for constructing an F_2 population. This F_2 population was initially used for identifying molecular markers associated with loci conditioning resistance to soybean cyst nematode (SCN). Additionally, we used the same population for evaluating the association of molecular markers for protein and oil concentrations. Leaf tissues from each of the 200 F_2 plants, together with parents, were used for DNA extraction and marker analysis. All individuals were allowed to be selfed to generate $F_{2:3}$ families, which were used for seed protein and oil concentration analysis.

Twenty-five grams of seed from each of the $F_{2:3}$ populations were used for measuring the percentage of protein and oil. These analyses were performed using a near-infrared (NIR) Food and Feed Analyzer located at the USDA National Center for Agricultural Utilization Research, Peoria, Illinois.

A total of 216 restriction fragment length polymorphism (RFLP) probes were used for screening both parents, Peking and Essex. The desirable clone/enzyme combinations from the screening were used for DNA hybridization to all 200 $F_{2:3}$ individuals.

Regression analysis was performed to determine the association of RFLP markers and loci controlling protein and oil concentrations. The proportion of the phenotypic variance explained by the segregation of the marker was determined by R^2 value.

Results and Discussion

The mean and coefficient of variation of the protein and oil concentrations for the two parents and their $F_{2:3}$ individuals are listed in Table 1. Compared with the parents, the $F_{2:3}$ progenies have far greater range of trait values. This transgressive segregation behavior suggests that the parents might have different alleles controlling oil and protein concentrations. Thus, the high genetic diversity between Peking and Essex may allow their progenies to be valuable source of new germplasm in breeding varieties with desirable protein and oil concentrations.

Restriction fragment length polymorphism marker B72 was found to be significantly linked to loci conditioning protein concentration (R^2 = 20.1%, $p < 0.005$), and another RFLP marker B148 was also associated with protein concentration (R^2 = 11.3%, $p < 0.003$). These two markers together explain 31.4% of the total phenotypic variation. Marker A715 was shown to be linked to loci governing oil concentration with R^2 = 7.0% and p = 0.01 (Table 2). In our previous study, RFLP marker B72 was also found to be linked to resistance loci for soybean cyst nematode race isolates 1 and 3 (data not shown), but the regression coefficient (R^2) between protein concentration and SCN resistance was not statistically significant.

Mansur et al. (1993) reported that RFLP marker K1 was linked to loci controlling oil concentration, and maker L48 was associated with loci underlying soybean protein concentration in the F_5 population, which was derived from a cross between 'Minsoy' and 'Noir 1'. We used a different population, which was generated from Peking x Essex, and found different RFLP makers, B148 (in LG - F) and B72 (in LG - H), linked to loci controlling soybean protein concentration, and A715 (LG - M) associated with loci conditioning oil concentration (Table 2). Data showing different molecular markers in different populations may indicate population specificity of DNA markers for polygenic traits. It may also be suggested that there are several DNA fragments linked to loci controlling the percentage of oil and protein.

In this study, soybean seed protein concentration was found to be inversely correlated with seed oil concentration, with a correlation coefficient r = -0.886 and p = 0.0001 (Table 3). This result is consistent with the data from another study by Lark, *et al.* (1994).

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Table 1. Mean and coefficient of variation (CV) of soybean oil and protein concentrations in parents and $F_{2:3}$ individuals.

Parents and progenies	Oil (%)		Protein (%)	
	Mean	CV	Mean	CV
Peking	16.40	---	40.56	---
Essex	19.40	---	42.70	---
$F_{2:3}$	18.63	0.16	42.23	0.08

Table 2. RFLP markers linked to protein and oil concentrations.

Traits	Markers	LG[†]	R^2 (%)	p <
Protein	B148	F	11.3	0.003
Protein	B72	H	20.1	0.005
Oil	A715	M	7.0	0.010

[†]: Soybean genome linkage group

Table 3. Correlation analysis of soybean protein and oil concentrations.

	Protein vs oil	p <
Correlation coefficient (r)	-0.8860	0.0001
Regression coefficient (R^2)	0.7850	0.0020

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Comparison of mitochondrial organization of four soybean cytoplasmic types by restriction mapping

Introduction

The limited germplasm base of modern domestic soybean cultivars (Delannay *et al.*, 1983) has made identifying sources of diversity an important aim of soybean crop improvement efforts. Molecular analyses of both chloroplast and mitochondrial genomes have been used to examine soybean diversity (Sisson *et al.*, 1978; Shoemaker *et al.*, 1986; Close *et al.*, 1989; Lee *et al.*, 1994). A unique 2.3 kb *Hin*dIII mitochondrial DNA fragment from 'Williams 82' was used as a cytoplasmic marker to classify soybean cultivars into four cytoplasmic types named for prototype varieties: `Bedford', Soja-Forage, `Arksoy', and `Lincoln' (Grabau *et al.*, 1989; Grabau *et al.*, 1992; Hanlon and Grabau, 1995). *Hin*dIII digestions and Southern blot analyses of mitochondrial DNA from the four cytoplasmic types with the 2.3 kb *Hin*dIII marker probe showed bands of 10.5 kb (doublet), 6.8 kb, 3.8 kb, and 2.3 kb, respectively.

In order to characterize mitochondrial genome rearrangements involved in the possible origin of the cytoplasmic types, we subcloned a 4.9 kb *Pst*I fragment containing the 2.3 kb *Hin*dIII sequence from a Williams 82 mitochondrial DNA cosmid library and determined its sequence (Hanlon *et al.*, 1997). The sequence of the 4.9 kb *Pst*I fragment contains short regions of homology to other known mitochondrial genes as well as to repeated sequences. Repeats are typical of higher plant mitochondrial DNA genomes and are potential sites for recombination. Due to the absence of any significant open reading frames, no obvious protein coding function could be assigned to this region. For the current report, subclones of the 4.9 kb *Pst*I region were used as hybridization probes to construct comparative restriction maps of the four cytoplasmic types in the polymorphic regions. Mapping data were compared to results of previous studies of soybean mitochondrial genome organization reported by Moeykens *et al.* (1995).

Materials and Methods

A cosmid library of Williams 82 mitochondrial DNA (Grabau 1985) was screened for homology to the 2.3 kb *Hin*dIII fragment. A 4.9 kb *Pst*I fragment containing the entire 2.3 kb *Hin*dIII sequence was subcloned into the plasmid pTZ19R. Four subclones of the *Pst*I fragment were isolated for use as hybridization probes: 1.7 kb *Pst*I-*Hin*dIII, 1.5 kb *Hin*dIII-*Xba*I, 0.8 kb *Xba*I-*Hin*dIII, and 0.9 kb *Hin*dIII-*Pst*I (see Fig. 1). Soybean DNA was isolated as described by Dellaporta *et al.* (1983) with the addition of a phenol:chloroform extraction step prior to ethanol precipitation. Restriction digests were performed with four restriction endonucleases (*Eco*RI, *Hin*dIII, *Pst*I, and *Xba*I), DNA fragments were separated by electrophoresis in 0.75% agarose gels, and bands were visualized under UV light by ethidium-bromide staining. DNA transfer to nylon membranes was performed by capillary blotting and hybridization procedures were carried out according to manufacturer's specifications (Micron Separations, Inc.).

Results and Discussion

Comparative restriction mapping of regions identified by the 2.3 kb *Hin*dIII marker was undertaken to identify potential sites of rearrangement among the four soybean cytoplasmic types. Digests of four representative cultivars were hybridized with the four subclones of the 4.9 kb *Pst*I mitochondrial fragment as probes (shown in Fig. 1) to construct partial restriction maps. The restriction maps showed one segment in common to all four cytoplasmic types, with the remainder of the mapped regions differing substantially among the four types. The common region is located at the far right-hand side of each map and is designated by the solid black bar. The exact nucleotide at which the common sequences diverge is unknown but Southern blotting data indicated that the common sequence extends from the right-hand *Pst*I site at least as far as the *Xba*I site. The common region is also repeated elsewhere in the mitochondrial genomes (data not shown). The common region has been sequenced as part of the 4.9 kb *Pst*I fragment (Hanlon *et al.*, 1997) from Williams 82 (Lincoln-type cytoplasm) which is designated by a line over the Lincoln restriction map in Figure 1.

Extensive restriction mapping of soybean mitochondrial DNA by Moeykens *et al.* (1995)

showed four copies of a 4.8 kb repeat in 'Illini' (Bedford-type cytoplasm), present on *Hin*dIII fragments of 11.2 kb, 10.2 kb, 8.7 kb and 7.7 kb. The location of a repeat in four genomic environments is a hallmark of recombinationally-active repeated sequences. The organization of the Illini sequences included four combinations of two left-hand flanking sequences (A and B) and two right-hand flanking sequences (C and D) on either side of the 4.8 kb repeated region. Our restriction map of Bedford DNA shows two alternative left-hand ends, indicated on the map as regions A and B, in order to remain consistent with the nomenclature used by Moeykens *et al.* (1995). The maps appear to encompass the 11. 2 and 10.2 kb *Hin*dIII Illini fragments mapped by Moeykens *et al.* (1995). The two *Hin*dIII fragments were identified in our previous studies as a doublet at approximately 10.5 kb. The location of the Illini 4.8 kb repeat is underlined on our Bedford restriction map (Fig. 1). The region corresponding to the C flanking region of Illini is designated by a line above the Bedford restriction map.

The proposed derivation of 6.5 kb, 3.8 kb, and 2.3 kb fragments by recombination with other sequences in the soybean mitochondrial genomes (Moeykens *et al.* 1995) is also consistent with our restriction mapping data for Soja-Forage, Arksoy, and Lincoln mitochondrial DNA (Fig. 1). The 6.5 kb *Hin*dIII fragment from Moeykens *et al.* (1995) corresponds well to the 6.8 kb size estimate in our studies. The data reported here extend the restriction mapping of the C flanking region beyond the *Hin*dIII site.

These studies confirm that the polymorphism we identified in soybean mitochondrial genomes with the 2.3 kb *Hin*dIII fragment also identifies a region adjacent to the 4.8 kb repeated sequence in Illini (Moeykens *et al.*, 1995). In addition, another repeated sequence lies at the right end of the C flanking region. The restriction maps provide an expanded view of the comparative genome organization flanking a sequence in common to the four cytoplasmic types. Sequence of one copy of the common region (contained within the 4.9 kb *Pst*I fragment) showed no obvious protein coding function as determined by the lack of an open reading frame. However, this region is conserved within the soybean mitochondrial DNA, suggesting that the region may play an important role in mitochondrial function or genome structure.

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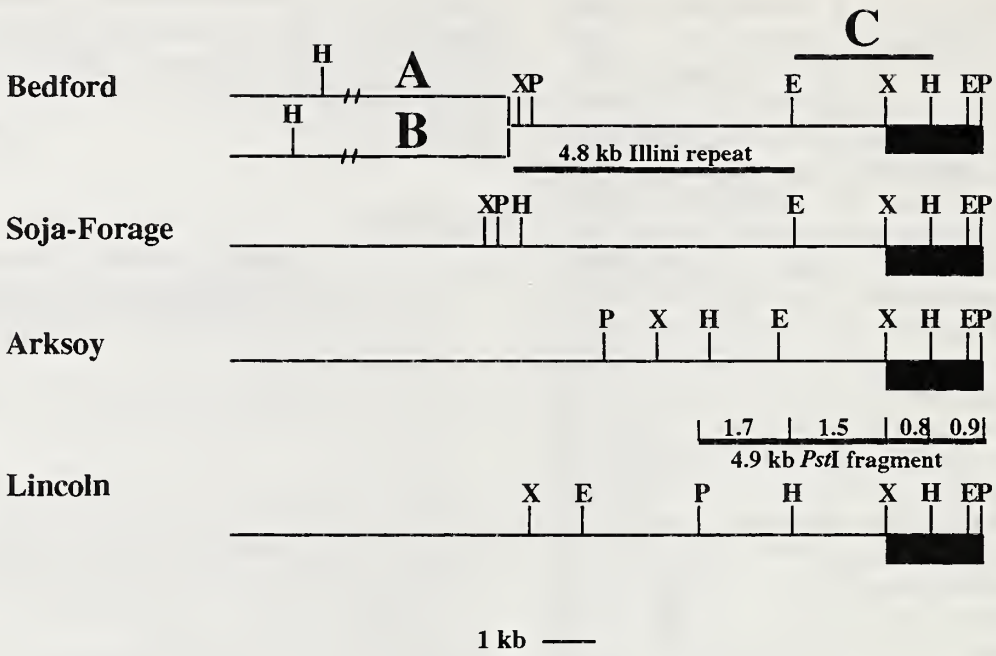

Figure 1. Partial restriction maps of mitochondrial DNA from four soybean cytoplasmic types containing the polymorphic regions originally identified by the 2.3 kb *Hin*dIII probe. The common region in each cytoplasmic type is indicated by the solid bar. The 4.8 kb Illini repeat described by Moeykens *et al.* (1995) is indicated by a line under the Bedford map. Moeykens *et al.* (1995) proposed two possible flanking regions on either side of the 4.8 kb repeat. A and B correspond to the two left-hand flanking regions in our maps. C represents one of the two possible right-hand regions flanking the repeat and is indicated by a line above the Bedford map. Approximate locations of restriction endonuclease sites are indicated by the following: E=*Eco*RI, H=*Hin*dIII, P=*Pst*I, X=*Xba*I. The 4.9 kb *Pst*I fragment, shown as a line above the Lincoln map, was isolated and sequenced from Williams 82 mitochondrial DNA. Subclones of this fragment used as probes in the study are indicated on the line above the Lincoln map: 1.7 kb *Pst*I-*Hin*dIII, 1.5 kb *Hin*dIII-*Xba*I, 0.8 kb *Xba*I-*Hin*dIII, 0.9 kb *Hin*dIII-*Pst*I.

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Location by Genotype Interaction Effects on Chemical Composition of Immature Large Seeded Vegetable-type Soybeans

Introduction

Due to changes in the dietary habits of American consumers across the United States, there is a greater demand for nutritious and healthy foods. With increased production and enhanced marketing, immature vegetable soybean may meet this demand. Vegetable soybeans are characterized by high protein content compared to other vegetable crops and are an excellent source of unsaturated fatty acid including the omega-3 factor. In addition, they are rich in most vitamins, and essential minerals (Mohamed and Rangappa, 1992).

Vegetable soybean (*Glycine max* (L.) Merr) genotypes generally fall into two categories: large-seeded and small-seeded. The large seeded garden types are used in fresh markets in urban areas with large oriental populations. Whereas, small-seeded types are used to prepare soy sprouts (Anonymous, 1987). Vegetable soybean is served in the pod as snack food, frozen, and as a vegetable mix. At present, immature soybean is imported into the United States to meet the demand for oriental speciality food products.

The protein and oil content of soybean seeds are not only conditioned by their inheritance, but also are influenced by the environmental conditions where the crop was grown (Xiangxun et al., 1991; Hue et al., 1990; and Taira and Taira, 1971). Protein and oil content of soybean vary among varieties as reported earlier (Mohamed et al., 1990, 1991, and 1992). Furthermore, the same variety or genotype in various years or various locations has shown significant differences. The amount of phytic acid varies from 0.4 to 2.06% in legumes and from 2.00 to 5.20% in oil seeds except soybean (Ready et al., 1981; Mohamed, 1991). In many cases, the phytic acid content is not considered to be absolute and may vary depending upon the genotypes, climatic conditions, locations, irrigation conditions, type of soil, and the year during which they grown.

One of the objectives of the Regional Soybean Research Project is to evaluate the nutritional quality of immature vegetable soybean and how it is affected by environmental conditions. Therefore, the following study was conducted to determine the effects of location by genotype interaction on the chemical composition of immature soybean seed.

Materials and Methods

Fourteen large-seeded vegetable soybean genotypes were used in this study. The seed size was determined based on weight/100 seeds. The selected genotypes had 20 gram/100 seeds or higher. Each entry was planted in four-row plots, arranged in randomized complete block design, at the research farms of Virginia State University (Petersburg, VA), Alabama A&M University (Normal, AL), Fort Valley State College (Fort Valley, GA), University of Maryland Easter Shore (Princess Anne, MD). The plants were harvested at reproductive stage R6, as described by Fehr et al. (1971). The harvested materials were immediately put in plastic bags and brought to the laboratory. Pods were removed from the harvested plants by hand. At each location, green immature seeds were separated from the pods, and kept frozen until all samples were collected. The samples were analyzed for total crude protein, oil, and moisture content as described in detail in our earlier reports (Mohamed et al., 1990, 1991). Total soluble sugars were extracted using 80% ethanol. Sugar content was then determined using the phenol method as described by Dubois et al. (1956). Phytic acid was extracted and determined according to Mohamed et al. (1996). Data were statistically analyzed and means were separated, by using Least Significant Difference (LSD) test at the 5% level of significance.

Results and Discussions

The analysis of variance of the data indicated that the effects of genotypes was highly significant for oil, total soluble sugars, moisture, and phytate. Except for protein, the effect of location on the analyzed parameters were highly significant (Table 1).

The immature soybean seeds showed significant differences in oil, sugars, phytate, and moisture, but no significant difference was found among genotypes for protein content (Table 2). Mean moisture content was 55.5 % and ranged from 51.3 for Akiyoshi to 60.2% for Mian Yan. These values are significantly lower than those reported earlier by Mohamed et al. (1992) for 23 Chinese

vegetable genotypes. These may be due to varietal or environmental differences. Mean percent protein was 32.8 and ranged from 32.2 for Ware to 34.4% for Tomahamare. No significant difference was recorded among genotypes, but highly significant differences were found between locations (Table 1). These genotypes had higher protein content than those reported earlier for Chinese vegetable soybean genotypes. Mean percent oil was 4.8% and ranged from 4.1 for Tanbagura to 6.0 % for Mian Yan. Similar to mature soybean seeds, a highly significant and negative correlation was found between oil and protein (r=-0.263**).

Significant variations among genotypes and between locations for total soluble sugars are documented. Mean soluble sugar was 11.67 mg/g seed and ranged from 10.4 for Hutcheson to 13.1 for Houjak. The higher sugar contents is a desirable characteristic for immature soybean seeds because of it's effect on the sweetness. Location x genotype interaction was highly significant which indicates that environmental conditions significantly affects sugar contents and this is similar to the results we found in mature seed planted at different locations.

Phytate is considered an anti-nutritional factor due to its effects on the availability of trace minerals, however, recent findings indicated that it plays an important role in the prevention of colon cancer. Mean phytate was 1.11 mg/g seed and ranged from 0.92 for Houjaku to 1.35 for PI 200506. Variations between locations was highly significant. Highly significant and negative correlations (r= -0.310**, -0.396**, -0.392**, and -0.319**) were found between location, oil, sugars, and moisture, respectively (Table 3).

In conclusion, the high protein, moderate sugars, oil contents, and lower phytate, may recommend the consumption of immature soybean seeds as a fresh food with high nutrient density. The forgoing discussion demonstrated that variation existed among genotypes for oil, sugars, phytate, and moisture, but not for protein.

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Table 1. Analysis of variance of total protein, oil, moisture, total soluble sugar, and phytate of selected large seeded soybean genotypes.

Source of Variance	df[a]	Mean square				
		Protein	Oil	Sugar	Phytate	Moisture
Genotype	13	7.14	3.29**	9.51**	0.187*	79.69**
Location	3	141.43**	22.76**	60.26**	2.600**	2457.72**
Genotype X location	39	7.85	2.59**	11.11**	0.212**	142.70**
Error	150	9.93	0.480	1.806	0.091	25.926

*,** Significant at the 0.05 and 0.001 probability levels, respectively.

[a] Degree of freedom

Table 2. Crude protein (16% Nitrogen), oil, moisture, total soluble sugar, and phytate contents of selected large seeded immature vegetable soybean genotypes.

Genotypes	Protein %	Oil %	T. S. sugar mg/g	Phytate mg/g	Moisture
Houjaku	32.52	4.97	13.12	0.921	53.37
PI - 200 506	34.20	4.70	12.37	1.345	52.24
PI - 417 427	32.27	4.45	10.57	1.081	52.52
PI - 416 981	33.02	4.300	12.45	1.097	54.33
Guanun Da Kei Dun	32.18	4.69	11.56	1.187	55.86
Shangrao Wan Gingsi	32.35	4.66	12.20	1.126	56.89
Akiyoshi	33.21	4.44	11.47	1.060	51.37
Tomahamare	34.42	5.43	11.51	1.056	58.40
Tousan - 122	32.04	4.54	11.15	1.170	56.73
PI - 181 565	32.96	5.20	11.45	1.252	56.86
Hutcheson	33.11	4.88	10.38	0.988	56.55
Ware	32.18	4.65	11.26	1.185	52.45
Mian Yan	32.28	5.97	11.03	1.018	60.22
Tanbagura	33.12	4.13	10.64	1.027	56.68
Mean	32.836	4.790	11.51	1.1159	55.38
C.V. %	9.60	14.47	11.67	26.96	9.19
LSD (< 0.05)	2.298	0.505	0.980	0.219	3.712

Table 3. Simple linear correlation coefficients among analyzed parameters of selected vegetable-type soybean.

Parameter	Location	Oil	Protein	Sugar	Phytate	Moisture
Location		-0.310**	0.204*	-0.396**	-0.392**	-0.319**
Oil			-0.263**	0.286**	0.139*	0.165*
Protein				-0.215**	0.068 NS	0.034 NS
Sugar					0.187**	0.029 NS
Phytate						0.074 NS

NS, *, ** Not significantly different or significantly different from zero at 0.05 and 0.01 probability levels, respectively.

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Stability Analysis of Vegetable Soybean

Introduction

In plant breeding and in cultivar trial programs genotypes are often evaluated for yield and quality traits in different environments prior to the selection or recommendation of certain genotypes. The presence of genotype x environment (GE) interactions may complicate the evaluation procedure and reduce the efficiency of genetic advance through selection. Selection of crop varieties for commercial cultivation based on phenotypic performance or yield per se is not much effective due to the presence of GE interactions. Regression analysis technique (Hill, 1975; Eberhart and Russell, 1966; Ng et al., 1980) is found to be useful to identify varieties for commercial and also for hybridization. Limited information is available on this aspect in vegetable-type soybean and suitability of regression analysis techniques has not been adequately tested for selection of parents to utilize in hybridization. Hence an attempt was made in this study to identify the desirable genotypes for hybridization program and also for commercial cultivation.

Materials and Methods

Ten vegetable-type soybean genotypes selected based on seed size were planted during 1993, 1994, and 1995 growing seasons at Randolph Research Farm of Virginia State University, Petersburg, Virginia in three planting dates (April 30, May 15, and May 30) in four-row plots arranged in split plot. Planting date was considered as a main plot and genotype as sub-plot. Each four-row plot was 3.6 m long, with a spacing of 75 cm between rows and a seeding rate of 23 seeds per meter. Conventional tillage practices were used and following soil test recommendations fertilizers were applied to the soil.

Each genotype was evaluated at green pod stage (between the R6 and R7, Fehr et al., 1971) by harvesting whole soybean plants from one meter length of the two center rows of each plot. The harvested materials were immediately put into plastic bags and brought to the laboratory. Pods were removed from the harvested plants, weighed and presented as green pod yield kg ha^{-1}. Pod samples were taken and 100 pod weight was recorded. The data were further analyzed for stability parameters according to the procedures outlined by Eberhart and Russell (1966).

Results and Discussion

Significant year-to-year variations were observed for green pod yield and hundred pod weight in the analysis of variance for the data combined over years. The genotypic variations were significant for both traits. These indicated that genetic variation existed for green pod yield and hundred pod weight among the genotypes tested and that selection could be effective for these traits. A significant genotype x year interactions (G x Y) were present for both traits measured. The significant interactions observed suggest that the performance or response of the genotypes used in this study were not stable from one season (year) to another. Therefore, multiple-year testings are required to assess accurately the genotypic potential of the genotypes.

The pooled analysis of variance revealed that mean differences between the genotypes and the year linear were significant for green pod yield and hundred pod weight when tested against pooled error (Table 1). There were also significant differences for green pod yield and hundred pod weight when the genotypes and years were tested against pooled deviation. These differences indicated the presence of variability among the genotypes as well as the season selected. The existence of significant G x Y interaction was shown by the item year + (G x Y) in the same table. Mean squares due to year plus G x Y interaction revealed that the genotypes interacted considerably with seasonal conditions. On partitioning the G x Y interaction, the linear component and pooled deviation (non linear) were significant for green pod yield and hundred pod weight when tested against pooled error (Table 1). There were also significant G x Y linear difference for hundred pod weight when tested against pooled deviation. This significance indicated that linear component of the G x Y is more important than the non linear for this trait. Thus, in these cases the responses of genotypes to varying seasonal conditions could be easily attributed to differences in regression slopes, indicating an almost accurate predictability of their phenotypic performance. However, variation due to linear components was not significant for green pod yield when tested against the pooled deviation, indicating that both linear as

well as non linear component of G x Y interactions were important. Finely and Wilkinson (1963) considered linearity of regression as a measure of stability. Eberhart and Russell (1966), however, emphasized that both linear (b_i) and non-linear (S^2_{di}) components of G x Y interaction should be considered in judging the phenotypic stability of a particular genotype.

Green Pod Yield:

The parameters of stability namely mean, regression coefficient and deviation from regression revealed that the genotypes Ware and AGS 129 were desirable genotypes with high mean yield level and regression coefficient less than one, indicating above-average stability and wider adaptability. These genotypes could be selected as parents for a hybridization program aimed at improvement of yield and also for commercial cultivation in order to stabilize yield. On the other hand PI 417310 has higher yield than the overall mean yield and regression coefficient greater than 1.0 (Table 2). This genotype was sensitive to changing environments with yield level higher than the overall mean. Hence, PI 417310 could be recommended only for high management conditions (favorable environments). The rest of the genotypes having below average yield level were not found suitable for commercial cultivation, but the genotypes AGS 129 and Ware could be used in breeding programs to develop high yielding and stable genotypes.

Among the ten genotypes tested, the cultivar Ware exhibited very high phenotypic stability and mean yield of 16,897 kg ha^{-1}. These data indicated that Ware has specific adaptation to low yielding environment since its pod yield is expected to be stable in most environments. The genotype AGS 129 also showed high phenotypic stability and mean yield equal to the grand mean. In the present material no strong association between phenotypic stability and mean performance was observed as reported by Finlay and Wilkinson (1963), although, it was noticed that the majority of the genotypes which exhibited below average phenotypic stability, gave above average mean yield. A similar negative relationship between stability and mean performance has also been reported by Eberhart and Russell (1966). In our study, the mean performance was independent of the linear and deviation from the linearity for both traits studied. Thus, in these cases the responses of genotypes to varying seasonal conditions could be easily attributed to differences in regression slopes, indicating an almost accurate predictability of their phenotypic performance.

Hundred Pod Weight:

The mean hundred pod weight of the genotypes was 125 g which ranged from 98 g for PI 417322 to 171 g to PI 417159. Among the genotypes tested PI 417322, Ware, Late Giant, and AGS 290 had lower mean hundred pod weight than the overall mean. Moreover, other genotypes that have shown higher mean hundred pod weight than the overall mean were PI 417159, Green & Black, PI 417213, and PI 417310. Only AGS 129 had mean hundred pod weight equal to the overall mean. The PI 417159 exhibited specifically high mean yield coupled with above average phenotypic stability which indicated that this is adapted to low yielding environments, since its hundred pod weight is expected to be stable in most environments. The genotypes AGS 290 and PI 417322 which had shown very high green pod yield and average phenotypic stability were observed to have hundred pod weight above and below average phenotypic stability, respectively. Other genotypes, PI 417213 and Green & Black in spite of their high hundred pod weight, their phenotypic stability is below average indicating these genotypes are adapted to a favorable environment.

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Table 1. Joint regression analysis of green pod yield and hundred pod weight of ten soybean genotypes used in the stability analysis.

Source of variation	df	Mean square	F (PEM)¶	F (PDMS)¶
		Green pod yield kg ha^{-1}		
Genotype (G)	9	12421127.9	6.1**	1.7
Year (Y) linear	1	3321986454.1	1618.6**	456.5**
Year + (G x Y)	20	175517376.6	85.5**	24.1**
G x Y (linear)	9	12844060.7	6.3**	1.8
Pooled dev	10	7276453.1	3.5**	-
Pooled error	330	2052360.8	-	
		Hundred pod weight(g)		
Genotype (G)	9	1305.1	22.1**	4.0*
Year (Y)linear	1	9702.6	64.1**	24.9**
year + (G x Y)	20	1005.4	17.1**	3.1*
G x Y (linear)	9	7115.9	120.4**	21.6**
Pooled Dev	10	329.0	5.6	-
Pooled Error	330	59.1	-	

¶ PEMS = Pooled error means squares; **PDMS** = Pooled deviation mean squares.

*, ** Significant difference at 5% and 1% probability levels, respectively

Table 2. Stability parameters for green pod yield (GPY) Kg ha^{-1} and hundred pod weight (HPW) for ten soybean Genotypes grown during 1993, 1994, and 1995 growing seasons

Genotype	GPY	b_i	S^2d	HPW	b_i	S^2d
Ware	16,897	0.84	1685501.33	118	0.53	-58.09
Green & Black	14,484	0.85	-2037222.57	155	0.59	1771.73**
AGS 129	15,152	0.92	963975.43	125	1.99	-15.70
PI 417213	13,923	0.85	6930087.21*	150	2.76	112.66
PI 416982	15,935	1.33	1096136.38	129	-0.09	-58.19
AGS 290	18,607	1.16	-2035167.10	124	0.41	-58.19
PI 417310	17,010	1.08	3672721.35	126	0.72	-48.28
PI 417159	16,511	0.70	18164540.92**	171	0.53	-404.92**
Late Giant	13,835	1.10	16794784.98**	114	0.60	83.15
PI 417322	17,963	1.17	005564.68**	098	1.94	565.34*
Mean	**15,965**			**125**		

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